



Research SPRING-8 FRONTIERS 2014

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PREFACE

Since its start of operation in 1997, SPring-8 has acquired a successful history as a progressive and innovative synchrotron radiation facility. Users of SPring-8 have carried out high-level research in a wide range of fields, including physics, chemistry, biology, medical science, and engineering, covering the basic research to practical applications. In 2013 and 2014, experiments carried out at SPring-8 resulted in 1002 and 822 published papers, respectively. In addition, user operation of the SACLA X-ray free electron laser facility was launched in 2012. In 2013, SACLA experiments resulted in 21 published papers, and the average number of citations per paper was as high as 8.3 in May 2015.



A number of SPring-8 and SACLA users were awarded prizes in 2014 and 2015 for their achievements in science and technology. Professor Hideo Hosono (Tokyo Institute of Technology) was awarded the Imperial Prize and Japan Academy Prize for his discovery and development of high-performance metal oxides as electroactive materials and catalysts. Professor Tetsuo Irifune (Ehime University) was awarded the Medal with Purple Ribbon for his research on ultra-high pressure synthesis in earth and materials sciences. The Medal with Purple Ribbon was also awarded to Professor Chikashi Toyoshima (The University of Tokyo) for his research on membrane proteins in the field of structural biology.

To maximize the scientific output and social impact of SPring-8 and SACLA experiments, we have recently reorganized the JASRI groups of beamline scientists and engineers into four divisions: Research & Utilization, Protein Crystal Analysis, Industrial Application, and XFEL Utilization. In addition, the Proposal Review Committee (PRC), whose role includes the beam time allocation for the 26 public beamlines of SPring-8, has been divided into eight subcommittees focusing on spectroscopy, XAFS/fluorescence analysis, diffraction/scattering, life science, long-term research, SR smart innovation, social interest, and industrial applications. The SACLA PRC decides the beam time allocation for SACLA users.

In this 2014 volume of SPring-8 Research Frontiers, two feature review articles are reported together with highlights of the scientific achievements of SPring-8 and SACLA users and beamline scientists. Two outstanding scientific reports based on SACLA experiments are included in the Chemical Science section, and twelve progress reports on the technology development in SACLA accelerators and beamlines are also included in this volume.

I am very grateful to the many authors and experts who contributed their papers to this volume. Special thanks are due to Dr. Naoto Yagi and the members of the editorial board for their constant efforts.

土肥 義治

Yoshiharu Doi
President
SPring-8/JASRI



EDITOR'S NOTE

SPRING-8 Research Frontiers 2014 covers outstanding scientific outcomes of SPRING-8 made mainly in the last two consecutive research periods, the second half of 2013 (2013B) and the first half of 2014 (2014A). Scientific achievements at SPRING-8 and SACLA in a wide range of basic and applied sciences including industrial applications are described. The development of accelerators, beamlines and experimental apparatus, and the present status of the SPRING-8 and SACLA facilities are also presented. In addition, the activities using NewSUBARU, which forms an integral part of the SPRING-8 research complex, are included.

This volume of SPRING-8 Research Frontiers has some different features from the previous ones. There are two review articles at the first part of the volume, which are on Earth Science and Cardiovascular Medicine. Advances in these fields made by using SPRING-8 in the last few years are summarized. It is planned to have such reviews for next several years to cover a variety of scientific activities at SPRING-8. I hope these reviews make clear the current position of SPRING-8 in each scientific field.

SACLA has been operating for three years now. In this volume, as was the case in last year, two outstanding scientific reports are included in Chemical Sciences. On top of these, the SACLA Accelerators & Beamlines Frontiers section of this volume contains twelve notable developments that have been made since SACLA became operational. As usual in a new facility, many new techniques have been developed and reported for SACLA, but these have not been included in the previous issues of SPRING-8 Research Frontiers. Thus, they are all included in this volume. These techniques have been developed for user experiments, so that, I would expect, there will be less articles in this section and more in the Scientific Frontiers sections in future. A similar transition from a technical development phase to an application phase indeed happened in the early days of SPRING-8, leading to a vast number of scientific outputs, as partly described in the two opening reviews. A similar course of development is anticipated in SACLA.

Some of the detailed information on the current and historical status of SPRING-8, such as the numbers of submitted and conducted proposals, budget, and publications, is available at the SPRING-8 website. (http://www.spring8.or.jp/en/about_us/spring8data/)

Copies of SPRING-8 Research Frontiers will be sent on request. The full text is also available on the SPRING-8 website (<http://www.spring8.or.jp/>). For the list of publications produced by SPRING-8 users and staff, please visit the publication database at http://www.spring8.or.jp/en/science/publication_database/.

We extend our appreciation to those who have recommended excellent research results suitable for publication in SPRING-8 Research Frontiers. We would also like to express our sincere gratitude to the users and staff of SPRING-8 for contributing their reports to this issue.

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SCIENTIFIC FRONTIERS

Review Article

Earth Science in SPring-8

Among the activities of SPring-8, researches in the field of Earth science plays an important role. In particular, two beamlines, BL04B1 and BL10XU, are equipped with state-of-the-art high-pressure and high-temperature apparatus and have been used to carry out numerous high quality works that had led to advances in Earth science in the last decade. In this review, we introduce some studies focused on the Earth's deep Interior.

1. Laser-heated diamond anvils at BL10XU

High-pressure and high-temperature *in situ* X-ray diffraction provides the most direct and reliable information on the minerals expected to exist deep inside the Earth. Such experimental study has only been made possible by the use of strong X-ray sources and synchrotron radiation to greatly expand the pressure and temperature ranges of the experiments. The first trial experiment of this nature was performed at the Photon Factory in Tsukuba in the 1980's using a large-volume high-pressure apparatus named MAX-80 [1]. Following the success of this apparatus, a new system was developed to extend the pressure to beyond 100 GPa that employed a diamond anvil apparatus combined with a laser heating system. This system was reproduced at SPring-8 and then further improved to take full advantage of the quality of the third generation source at SPring-8 [2,3]. Laser heating combined with the diamond anvil can heat samples to beyond 5000 K, although the heating spot is very small (typically less than 20 μm in diameter) and a very large temperature gradient exists in the heated area. The X-ray optics

at BL10XU was designed so that a very sharp and small monochromatic X-ray beam can be obtained. As a result, very high quality powder X-ray diffraction patterns can be obtained even under the extreme conditions corresponding to the deep part of the Earth.

1-1. Discovery of the post-perovskite phase of MgSiO_3 in the deep mantle of the Earth

It is well known that the upper part of the mantle of the Earth, the area down to about 400 km below the surface, mainly consists of three minerals: olivine (Mg_2SiO_4), pyroxene (MgSiO_3), and garnet ($\text{Mg}_3\text{Al}_2\text{Si}_3\text{O}_{12}$). It was discovered in the 1970's that pyroxene and garnet transform into much denser minerals with a perovskite structure, while olivine decomposes into a mixture of phases with perovskite and rock salt structures. Many studies have been carried out to clarify the stability limit of these silicate perovskites, and these studies suggested that the silicate perovskite is stable down to the bottom of the lower mantle, which extends a depth of 2900 km. Since the volume of the lower mantle

is more than half the entire volume of the Earth, the silicate perovskite is the most abundant mineral of our planet, even though we cannot observe this high-density mineral at the surface of the Earth.

Hirose's group at Tokyo Institute of Technology has been working intensively to clarify the behavior of various rocks under the conditions of the deep lower mantle, and they found unidentified diffraction peaks using the system at BL10XU. Further studies clarified that even for the very simple mineral MgSiO_3 (bridgmanite), the perovskite structure transforms into a new phase above about 120 GPa, which is only 10 GPa below the pressure at the core-mantle boundary. They summarized this work and published it with the title "Post-perovskite phase transition in MgSiO_3 " in *Science* (2004) [4]. This new phase of the silicate made it possible to solve various baffling problems arising from seismic studies of the core-mantle boundary region. Since then, numerous works have started to clarify the nature of this post-perovskite phase and the above-mentioned paper has been cited more

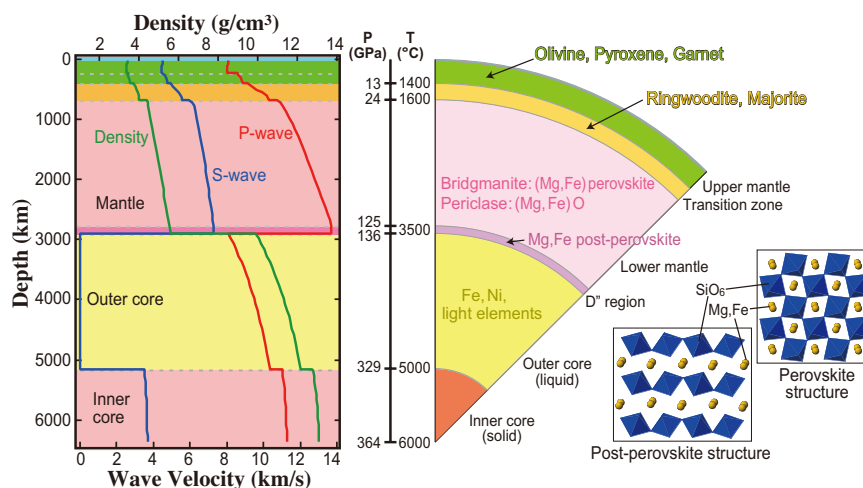


Fig. 1. Major minerals in the Earth.

than 800 times by researchers around the world. This clearly shows the huge impact of this work in the field of Earth science. Figure 1 shows the main minerals expected to exist inside the Earth. As is clear from this figure, the post-perovskite phase is the last and the most dense silicate mineral expected to exist in the Earth, and the research carried out at SPring-8 has played a key role in clarifying this new paradigm in Earth science.

1-2. X-ray diffraction study under conditions corresponding to the center of the Earth

Further efforts were made to extend the pressure and temperature ranges of powder X-ray diffraction studies to the deeper part of the Earth, the core, mainly by reducing the size of the sample. In 2010, Tateno *et al.* reported a stable phase of iron under the conditions at the center of the Earth [5]. At a pressure of about 360 GPa and a temperature of over 5000 K, they showed that the *hcp* phase is the stable phase of iron. Many groups worldwide have attempted to clarify the phase relation of iron, which is the main component of the core, and various results have been reported. Among them, the work of Tateno and his coworkers covered the highest pressure and temperature ranges and, as is clear from Fig. 2, the quality of the diffraction data was sufficiently high to convince many other scientists of the validity of their results. Therefore, so far as pressure and temperature conditions are concerned, it is now possible at SPring-8 to achieve conditions at any part of the Earth and to study in detail the stable phases of materials under these extreme conditions.

1-3. Efforts to further extend the pressure range

Efforts to extend the pressure range beyond that of the Earth are also being made using the double-stage diamond anvil technique. In the solar system and in the universe,

there are many places subjected to much higher pressures than the center of the Earth, such as the inside of Jupiter and Saturn. The behavior of materials above 500 GPa is also a very interesting and fascinating target for research in physics and chemistry, and many groups worldwide are attempting to further extend the pressure range of experiments. We employed the double-stage diamond anvil technique, described as follows. A very tiny anvil with a culet (a top flat part to generate a very high pressure) diameter of only 3 μm was prepared using a focused ion beam (FIB) that was developed to process very small samples for electron microscopy. As shown in Fig. 3, a pair of these tiny anvils was placed in the sample chamber of a conventional diamond anvil and squeezed with a sample between them. In this way, a high confining pressure is applied to these second-stage anvils, which is expected to extend the pressure range further. Although many technical problems still remain and the achieved pressure has not exceeded that of the single-stage diamond anvil technique, the results have high reproducibility and very promising results have been obtained [6]. In this study, the size of the powder sample was only a few microns in diameter and the thickness was less than one micron. As a result,

a very small and strong X-ray beam is required to obtain measurable diffraction from such a tiny sample. The quality of the X-ray optics of BL10XU is sufficiently high in terms of stability and brilliance to obtain good diffraction data from the small area of such a tiny sample.

1-4. Multiple-property measurements under extreme conditions

Although X-ray diffraction data to determine the crystal structure is the most basic information when investigating the properties of materials, information on many other physical properties is also desirable. The most basic and directly obtainable information about the deep interior of the Earth is provided by seismic

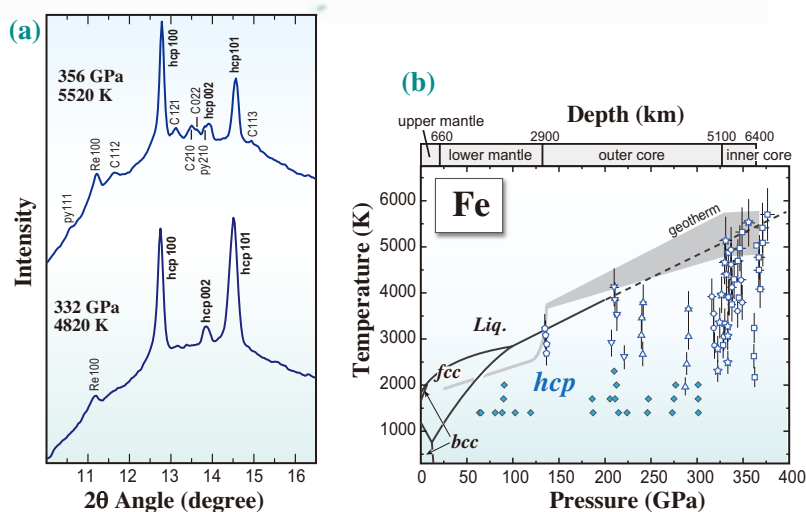


Fig. 2. (a) Diffraction spectra under the conditions at the center of the Earth and (b) phase diagram of iron.

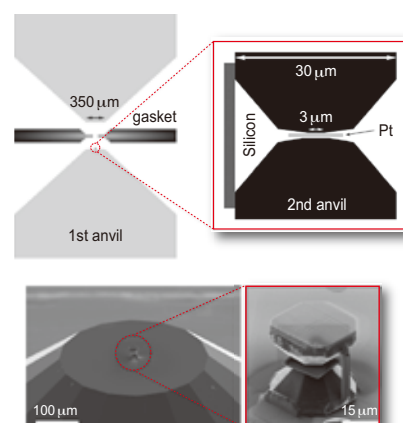


Fig. 3. Double stage diamond anvil.

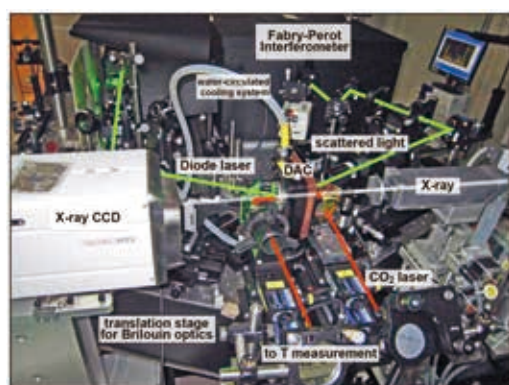


Fig. 4. Brillouin scattering setup.

observations. The propagation velocities of longitudinal and transverse waves through the whole of the Earth have been well studied. Using X-ray diffraction, however, we can obtain the crystal structure and density of minerals. By measuring pressure derivatives of the density, we can obtain elastic parameters related to longitudinal waves but no information can be obtained for transverse waves. Brillouin scattering is a powerful technique for directly measuring the elastic wave velocities of a tiny sample. At BL10XU, a new system for performing simultaneous measurements of X-ray and Brillouin scattering was constructed by Murakami *et al.* [7]. As shown in Fig. 4, the system is complicated because four probes, the X-ray beam for diffraction, the laser beam for Brillouin scattering, the laser beam for heating the sample, and visible light for sample observation have to be focused precisely at a tiny sample subjected to high pressures.

This system has made it possible to measure the elastic wave velocities of various materials over very wide pressure and temperature ranges and to obtain powder X-ray diffraction data simultaneously. Various studies have been carried out on MgSiO₃ perovskite [8], post-perovskite [9], and SiO₂ [10], and on the basis of these studies, important issues have been raised regarding the deep interior of the Earth.

2. Large volume high-pressure apparatuses at BL04B1

Two large-volume high-pressure apparatus, SPEED-1500 and SPEED-1500 Mark.II (Fig. 5), are installed at BL04B1 of SPring-8. They are driven by large hydraulic rams with a capability of 1500 tons and can compress a cubic or octahedral pressure-transmitting medium to a size of about 1 cm. Compared with the laser heated diamond anvil apparatus, the pressure and temperature range attainable using these apparatuses are rather limited, and routine studies have so far been carried out below 50 GPa. However, a much more well controlled and stable sample temperature can be achieved and the sample size can be on the order of a few mm, which is two orders of magnitude larger than that when using diamond anvils. These characteristic features are particularly important for studying multicomponent systems and mixtures of various minerals, i.e., rocks, which are the main component of the Earth. Efforts to increase the pressure beyond 50 GPa have been made and the successful generation of a pressure over 100 GPa was recently achieved using a Kawai-type multi-anvil high-pressure apparatus with large sintered diamond anvils [11]. At present, multi-anvil experiments at such high pressures are only possible at SPring-8. This technical breakthrough has contributed to the understanding of the Earth's entire mantle and is playing a major role in the growth of high-pressure research.

Since a sample under pressure is surrounded by many materials such as the capsule, heater, pressure-transmitting medium, and gasket, the X-ray beam has to travel through all these materials to reach the sample and the length of the propagation exceeds several centimeters. As

a result, a very high energy X-ray beam, typically more than 50 keV, is required for such experiments. Fortunately, SPring-8 can produce a high-energy white X-ray beam whose maximum energy exceeds 100 keV. Combined with a solid-state detector (SSD), high-quality X-ray diffraction data can be obtained in a short time, typically on the order of 100 s.

Another characteristic feature of this beamline is the use of X-ray radiography. By combining brilliant and parallel X-ray beam with a CCD camera, we can obtain a precise image of a sample under a high pressure and high temperature with a resolution of a few micrometers. Radiography observation can provide a variety of information difficult to obtain by other measurements. For example, by measuring the sinking velocity of a small platinum ball in a molten silicate, the viscosity of the molten silicate has been studied as a function of pressure. Another example is the simultaneous measurement of stress and strain under pressure.

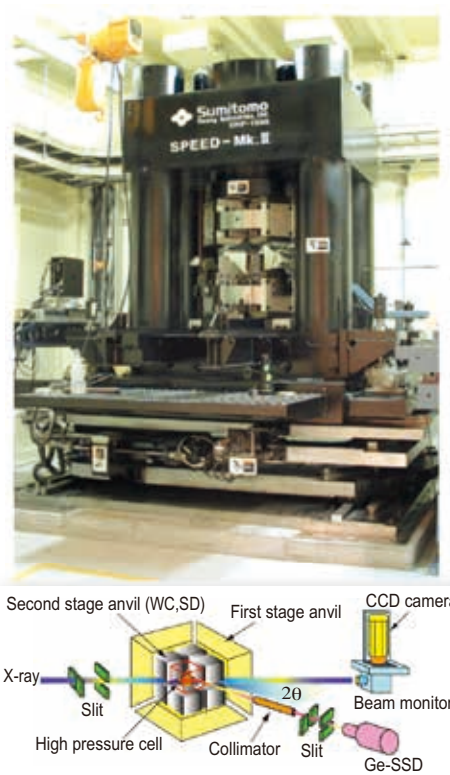


Fig. 5. Large-volume press and schematic illustration of the experimental setup.

Stress can be measured from the change in the unit cell volume using the powder X-ray pattern, while bulk strain can be measured by directly observing the bulk size of the sample by radiography.

Another characteristic feature of BL04B1 is the simultaneous measurement of X-ray and ultrasonic velocities of a sample. This allows us to precisely compare laboratory data and seismic observations of the Earth and we can make detailed argument about the composition and structure of the Earth's deep interior. Some examples of works performed using the above-mentioned features are introduced.

2-1. Detailed study on the transition zone (from 400 to 700 km depth) of the Earth

The transition zone is the area where various pressure-induced transitions occur in the minerals constituting the Earth, and both the sound velocity and density increase very rapidly in this area. Irifune's group performed simultaneous *in situ* velocity and X-ray measurements on garnets under the conditions of the transition zone using the systems at BL04B1 [12]. Using these data, they succeeded in clarifying that the chemical composition called the

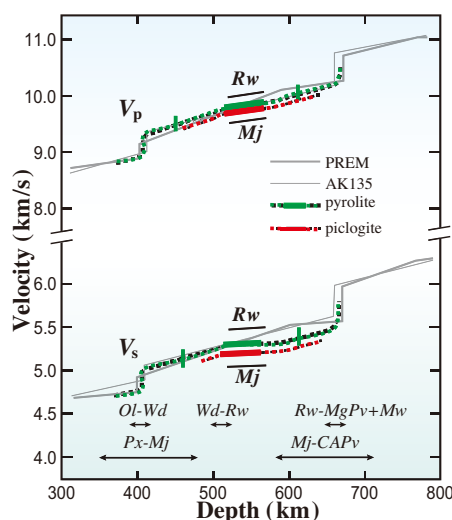


Fig. 6. Comparison of the sound velocities for pyrolite and piclogite compositions with representative seismological models.

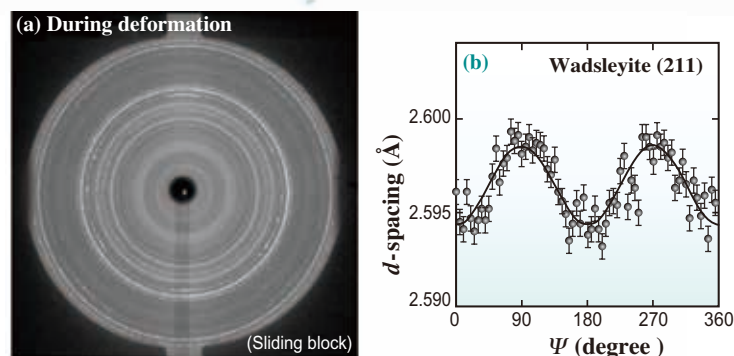


Fig. 7. (a) Typical X-ray diffraction pattern of wadsleyite and (b) variation of *d*-spacing with azimuth angle.

pyrolite model more convincingly explains the seismic velocity profile in this area than another competing model called the piclogite model (Fig. 6). Direct observation of the chemical composition at this depth is impossible and the systems at SPring-8 are becoming very powerful for such study.

2-2. Rheological study of rocks and minerals in the deep mantle

Not only the stable structures but also the dynamics is becoming increasingly important for obtaining a correct understanding of our planet. The most basic information for such studies is the rheological properties of the constituent materials, which are extremely difficult to obtain under very high pressures. Therefore, experimental studies have so far limited to the conditions of the shallow upper mantle. However, the systems developed by Kawazoe's group at BL04B1 have made it possible to extend such studies to the conditions of the transition zone [13]. By combining the capabilities of D-DIA high-pressure apparatus, X-ray radiography, and X-ray diffraction, they have succeeded in measuring the stress-strain relations of various materials under the conditions corresponding to the transition zone (Fig. 7). Such studies will greatly affect our understanding of the dynamics in the deep interior of the Earth.

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References

- [1] O. Shimomura *et al.*: "Solid State Physics under Pressure - Recent Advance with Anvil Devices", S. Minomura ed., TERRAPUB, Tokyo, (1985) 351.
- [2] T. Yagi *et al.*: Rev. Sci. Instrum. **72** (2001) 1293.
- [3] T. Watanuki *et al.*: Rev. Sci. Instrum. **72** (2001) 1289.
- [4] M. Murakami *et al.*: Science **304** (2004) 855.
- [5] S. Tateno *et al.*: Rev. Sci. Instrum. **86** (2015) 033905. (highlighted in Research Frontiers 2010)
- [6] T. Sakai *et al.*: Rev. Sci. Instrum. **86** (2015) 033905.
- [7] M. Murakami *et al.*: Phys. Earth Planet. Int. **174** (2009) 282. (highlighted in Research Frontiers 2007)
- [8] M. Murakami *et al.*: Nature **485** (2012) 90. (highlighted in Research Frontiers 2012)
- [9] M. Murakami *et al.*: Earth and Planet. Sci. Lett. **259** (2007) 18.
- [10] Y. Asahara *et al.*: Am. Mineral. **98** (2013) 2053.
- [11] D. Yamazaki *et al.*: Phys. Earth Planet. Int. **228** (2014) 262. (highlighted in Research Frontiers 2013)
- [12] T. Irifune *et al.*: Nature **451** (2008) 814. (highlighted in Research Frontiers 2008)
- [13] T. Kawazoe *et al.*: Am. Mineral. **96** (2011) 1665.

Cardiovascular Medicine in SPring-8

Cardiovascular diseases are the number one cause of death globally. To elucidate the underlying mechanisms of cardiovascular diseases, it is necessary to advance our understanding of structural and functional properties of the heart and vascular system under normal conditions as well as the dynamic changes under pathological conditions. SPring-8 provides several advanced experimental techniques for studying cardiovascular structure and function. This review focuses on three novel techniques: microangiography, X-ray diffraction, and high-resolution phase contrast tomography.

Microangiography

The resistance vessels of the microcirculation (~ 30 – $200\ \mu\text{m}$ diameter, arterioles-small arteries) are the target points of various cardiovascular diseases. The vascular tone of these vessels is meticulously modulated via multiple mechanistic pathways including the vascular endothelium and smooth muscle as well as neurohumoral stimuli to control the resistance to the flow and hence optimize blood flow at the local tissue and whole-organ levels. Impairment of any of these regulatory systems adversely affects the vascular control of blood flow with abnormal vascular constriction and remodeling and, thus, increases the likelihood of lethal events such as myocardial infarction, stroke and hypertension. For a better understanding of the control of blood flow, it is necessary to directly measure the internal diameter (ID) and flow velocity in resistance vessels.

X-ray contrast absorption imaging techniques, such as cineangiography,

have the advantage that X-rays pass through tissues more easily than light without exposing the organ of interest, making it possible to obtain a dynamic overview of the entire vascular network deep within an intact organ in real-time. Using conventional X-ray tubes, it is not currently possible to obtain images with exposure times of less than 10 ms and a spatial resolution better than $100\ \mu\text{m}$ [1]. Synchrotron radiation (SR) absorption microangiography has been developed for the high-spatial resolution (up to $4.5\ \mu\text{m}$ resolution) and high-speed (few milliseconds) imaging of resistance vessels by improving the single-energy temporal subtraction technique at beamline BL28B2 in SPring-8 [1]. SR has several distinct superior qualities compared with conventional angiography. In essence, SR is characterized by high brilliance

and extreme collimation (minimal divergence of X-ray photons), allowing greater image contrast and sharpness without magnification artifacts for vessels at different depths. The high X-ray intensity also allows a greater flux even during a short exposure time to produce blur-free images of the hearts of small animals with a high heart rate (>500 beats/min). Rapid imaging (1–2 ms shutter open times) permits the accurate analysis of vessel diameters in a beating heart based on individual image frames without artifacts due to organ motion. SR allows selective tuning of the X-ray energy (33.2 keV, slightly higher than the iodine *K*-edge energy) with a monochromator, maximizing the difference in photon absorption between an iodine contrast agent in the vessels and surrounding tissues.

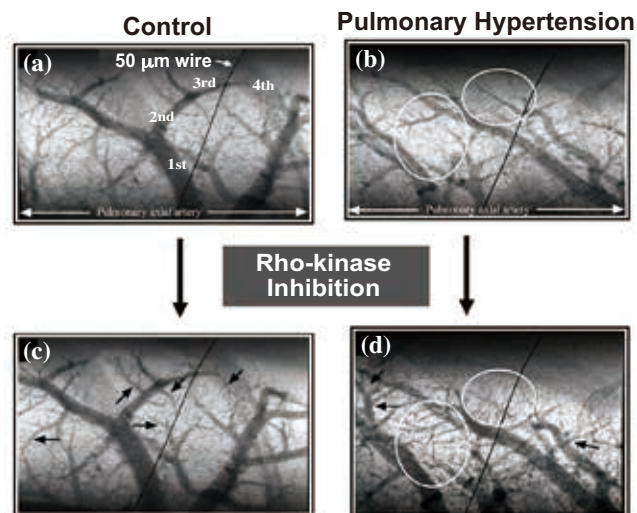


Fig. 1. Representative microangiograms showing the branching pattern of rat pulmonary arteries in control (**a, c**) and pulmonary hypertension (**b, d**) before (**a, b**) and following (**c, d**) Rho-kinase inhibition. The black arrows indicate pulmonary arteries that have dilated as a result of Rho-kinase inhibition. The open white circles in image (**b**) indicate regions with few opaque vessels, which subsequently, in image (**d**), appear to increase in vessel density following Rho-kinase inhibition, due to the reperfusion of previously constricted vessels. This was evident only in hypertensive rats.

SR microangiography has now been used for repeated visualization of the microcirculation in various organs such as the heart, lungs, brain, kidneys and hind limbs, especially in small animals [1,2]. SR allows us to identify resistance vessels with localized or nonuniform constriction and to characterize the extent of dysfunction of the vascular endothelium and smooth muscle in disease states. One prominent example is microangiography experimentation on rat models of pulmonary arterial hypertension [1,3,4], which is an intractable disease attracting much attention. The activation of Rho-kinase is increasingly recognized to be involved in the pathogenesis of this lethal disease. Recently, microangiography has revealed that the inhibition of Rho-kinase causes the dramatic emergence of pulmonary arterioles ($<100\ \mu\text{m ID}$) that were not visibly opaque in monocrotaline-induced rat pulmonary hypertension (Fig. 1) [3]. This appearance of “new” vessels indicates that severe Rho-kinase-mediated vasoconstriction restricts the pulmonary blood flow distribution by occluding the arterioles, which contravenes the long-held paradigm that pulmonary vessel rarefaction was the sole cause of vessel density reduction in pulmonary hypertension. It is very difficult to detect such regional microvascular dysfunction by using any other intravital methods including microscopy, CT and MRI.

High-resolution, high-speed angiography permits studies on a living mouse, and the coronary and pulmonary arterioles ($\sim 50\ \mu\text{m} < \text{ID}$) have been successfully visualized (Fig. 2) [1,5], allowing the analysis of genetic and molecular mechanisms for cardiovascular and pulmonary diseases at the whole-body level by using specific gene-targeted knockout and knockin mice. When vessel diameters are $<50\ \mu\text{m ID}$ and sequential images are not confounded by vessel movement, such as in the brain, image summation is also used

to enhance the vessel morphology. SR microangiography has shed new light on the control of blood pressure and flow in health and disease.

X-Ray Diffraction of Cardiac Muscle

Many techniques are used to investigate how cardiac performance is regulated at the sarcomeric level in the heart. However, none of these techniques directly examines the movement or activity of the contractile proteins in the beating heart. The most significant advantages of X-rays are its high penetration power, which is used in medical diagnosis, and its short wavelength, which enables us to investigate structures at the atomic level. Combining these advantages enable us to investigate interactions between contractile proteins in muscle cells [6].

There is a hexagonal lattice of contractile filaments in muscle cells (Fig. 3(a)). The regular arrangement with a spacing of 30–40 nm gives rise to X-ray diffraction spots called equatorial reflections (Fig. 3(b)). The position of the spots represents the distance between filaments, while their intensity is related to the location of myosin heads that span filaments to produce the contractile force. The measurement of these reflections provides information on the molecular events taking place in cardiac cells. To examine a beating whole heart, an X-ray with a high energy (15 keV) that has high penetration power is necessary. By exposing a whole heart to a finely collimated intense beam at BL40XU (dimensions, $0.2 \times 0.2\ \text{mm}^2$)

it is possible to record the diffraction peaks in a myocardial region without averaging over multiple heart beats. Although there are layers of muscle fibers running in different directions in the heart wall, it is possible to account for the diffraction pattern recorded at different angles and depths in the heart [7].

Open-chest heart preparation allows a researcher to investigate regional differences in cross-bridge dynamics because the myocardial regions exposed to the beam can be determined [8]. Ventricular pressure and volume can be measured simultaneously. Figures 3(c) and 3(d) show the intensity changes of the equatorial reflections together with left ventricular (LV) pressure-volume recordings of the heart. Figure 3(c) was obtained from a normal rat heart while Fig. 3(d) was recorded in the same region of the heart after 30 min of ischemia (caused by occlusion of the coronary artery) followed by the reperfusion of blood. The figures show how the myosin mass transfer (cyclic changes in intensity ratio) can be estimated from continuous short SAXS recordings and how this localized index is altered by an episode of ischemia-reperfusion, whereas the ventricular pressure remains preserved in the same heart.

Recently, using this X-ray diffraction technique on a rat, it was demonstrated that contractile proteins in cardiac muscle cells that were derived from iPS cells and transplanted to an infarcted heart regularly operate in synchrony with the host heart (Fig. 4) [9].

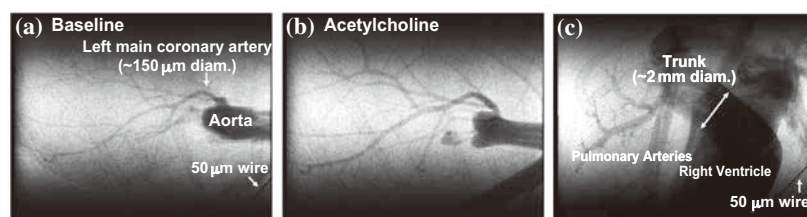


Fig. 2. Coronary and cardiopulmonary microangiograms of the intact chest in a living mouse. The small size of the mouse heart permits coronary imaging of the whole heart (a) and evaluation of endothelial stimulation (acetylcholine $5\ \mu\text{g/kg/min}$, b). Fast image acquisition of the mouse lungs enables visualization of multiple branching orders of pulmonary arteries and estimation of pulmonary blood flow transit time (c).

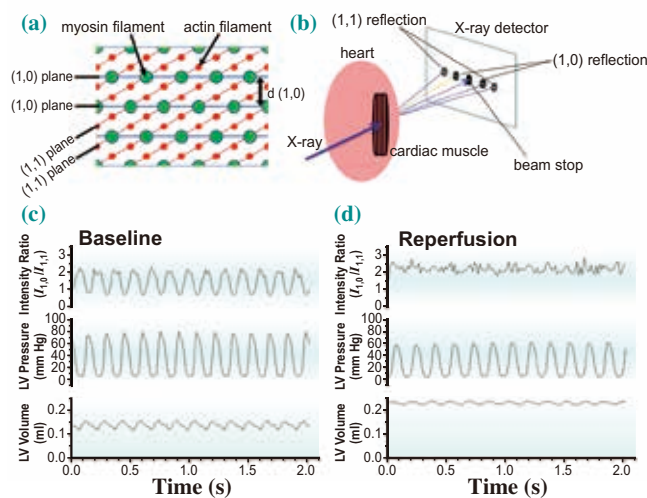


Fig. 3. X-ray diffraction from cardiac muscle. (a) Cross-section of the hexagonal lattice of myosin filaments (green) and actin filaments (red). In cardiac muscle, four diffraction peaks appear symmetrically at right angles to the filament lattice: the (1,0) and (1,1) reflections. (c) and (d), Time traces of intensity ratio ($I_{1,0}/I_{1,1}$), left ventricular pressure and volume.

Cardiac muscle regeneration with iPS cells is regarded as the next-generation cardiac therapy. This work provided important evidence for the development of next-generation therapy for cardiac diseases by combining two leading-edge technologies, iPS cells and SPring-8, which originated in Japan.

Phase-contrast Microtomography

Radiographs are formed by the attenuation of X-rays by an object. It has been pointed out that the phase shift of an X-ray by an object is much larger than its attenuation, and thus, phase-contrast imaging is more sensitive (up to 1000 times) to small density differences than absorption-contrast imaging [10]. To measure phase shifts for imaging, an optical device called an interferometer is required. A grating-based interferometer ("Talbot" type) (Fig. 5) is now commonly used, which has been adapted for SR by Momose [11]. Measurement with the interferometer provides phase shifts in a projection image. After tomographic reconstruction, the value of the X-ray

refractive index in each cubic voxel can be determined. As the refractive index differs much more than the absorption coefficient among different materials, a phase-contrast image has a higher contrast than an absorption-contrast image.

A grating-based interferometer is available at BL20B2 and BL20XU. It has been used on various soft tissues such as spinal cord [12], eye lens [13], kidney [14] and a fetus [15]. In the circulatory system, an atherosclerotic plaque was imaged successfully [16]. Recently, an aortic wall was studied extensively by phase-contrast CT at SPring-8. With improvements in the fast scanning technique, it is now possible to obtain phase-contrast tomographic images within 30 min, which has greatly facilitated studies on fresh samples. Hoshino *et al.* [17] showed the deformation of an aortic wall with various degrees of stretching (Fig. 6). This type of dynamic tomographic measurement has made it possible to study density changes in the wall with various degrees of stretching.

Phase-contrast tomography is generally useful for obtaining high contrast in images of soft tissues and organs. Figure 7 shows a slice of a whole human fetus heart obtained by phase-contrast tomography. Not only the muscle fibers but also the branching bundle of the conduction system is clearly observed in the

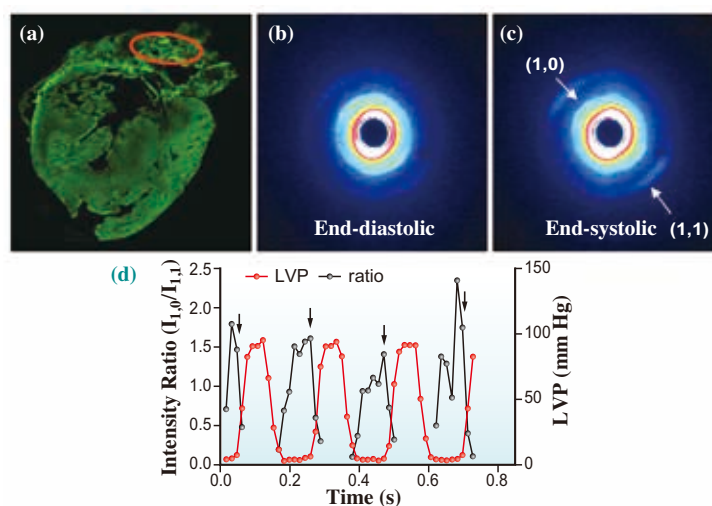


Fig. 4. (a) Small-angle X-ray scattering analysis of the red-circled area indicating the iPSC cell-derived cardiac myocytes (iPSC-CM) transplanted to an infarcted heart. (b) and (c) 1,0 and 1,1 equatorial reflections at the end-diastolic and end-systolic phases. (d) Myosin mass transfer index (equatorial intensity ratio $I_{1,0}/I_{1,1}$) in an iPSC-CM sheet and simultaneously acquired LV pressure over several consecutive cardiac cycles of an infarcted heart. Significant 1,0 myosin reflections were only evident for part of the cardiac cycle due to heart movement. When significant actin-myosin reflections were evident, the shift in the myosin mass towards actin (decrease in intensity ratio) coincided with the rapid increase in LV pressure during systole, showing synchronized contraction of the iPSC-CMs in the sheet. Arrows indicate the timing of end-diastole.

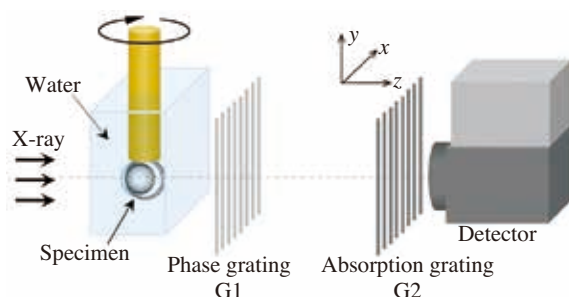


Fig. 5. Schematic diagrams of grating-based interferometer (for Talbot imaging). For each projection image, three to five images are recorded with different shifts of the G2 grating along the x-axis.

interventricular septum without any excision of the sample [18]. As knowledge of conduction system anatomy is critical to prevent complete heart blockage as a complication of repair, the application of phase-contrast tomography may help great progress to be made in evaluating the conduction system anatomy in the case of various congenital heart defects.

Future prospect

Despite the plethora of studies investigating the mechanisms for cardiovascular dysfunctions, the origin of most cardiovascular diseases is still unknown. This high prevalence of idiopathic

pathologies has stemmed, at least in part, from the inability to reliably assess the structural and functional properties of the heart and vascular system because of methodological constraints. The development of SR techniques in SPring-8 now holds out the promise of rapid advancement of our understanding of the cardiovascular structure and function. Since cardiovascular research requires a variety of experimental approaches, the combined use of SR techniques, not only those mentioned in this review, will further promote the elucidation of mechanisms and the development of better treatment and prevention of cardiovascular diseases.

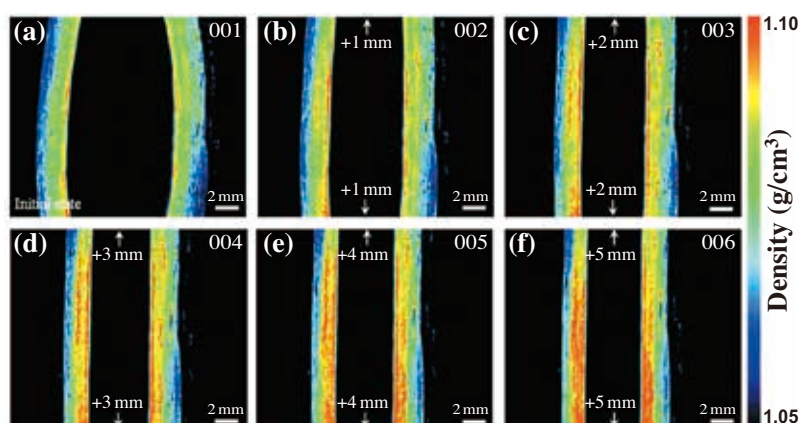


Fig. 6. X-ray phase contrast computed tomography images of a ring-shaped fresh pig aorta subjected to stretching. (a) Initial state. (b)–(f) Stretched by hooks by multiples of 2 mm (1 mm by the upper hook and 1 mm by the lower hook).

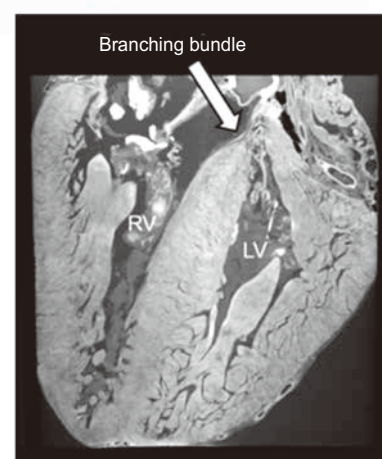


Fig. 7. Cross-sectional image of a whole fetus heart. The branching bundle is clearly observed in the interventricular septum. RV: right ventricle, LV: left ventricle. The formalin-fixed fetus heart was provided by Drs. Yoshihiro Oshima and Makiko Yoshida at Kobe Children's Hospital.

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References

- [1] M. Shirai *et al.*: *Circ. Res.* **112** (2013) 209.
- [2] M.J. Jenkins *et al.*: *Arterioscler. Thromb. Vasc. Biol.* **32** (2012) 370.
- [3] D.O. Schwenke *et al.*: *J. Appl. Physiol.* **110** (2011) 901.
- [4] D.O. Schwenke *et al.*: *Pflug. Arch. Eur. J. Physiol.* **462** (2011) 397.
- [5] T. Sonobe *et al.*: *J. Appl. Physiol.* **111** (2011) 75. (highlighted in Research Frontiers 2012)
- [6] I. Matsubara: *Ann. Rev. Biophys. Bioeng.* **9** (1980) 81.
- [7] N. Yagi *et al.*: *Biophys. J.* **86** (2004) 2286.
- [8] J.T. Pearson *et al.*: *Circulation* **109** (2004) 2983. (highlighted in Research Frontiers 2004)
- [9] T. Higuchi *et al.*: *Cell Transplant.* DOI: 10.3727/096368914X685799 (2015).
- [10] U. Bonse and F. Busch: *Prog. Biophys. Molec. Biol.* **65** (1996) 133.
- [11] A. Momose: *Opt. Express* **11** (2003) 2303.
- [12] K. Takashima *et al.*: *J. Synchrotron Rad.* **22** (2015) 136.
- [13] M. Hoshino *et al.*: *PLoS ONE* **6** (2011) e25140. (highlighted in Research Frontiers 2011)
- [14] J. Wu *et al.*: *Kidney Int.* **75** (2009) 945. (highlighted in Research Frontiers 2009)
- [15] M. Hoshino *et al.*: *Biol. Open* **1** (2012) 269.
- [16] M. Shinohara *et al.*: *Am. J. Physiol.* **294** (2008) H1094. (highlighted in Research Frontiers 2008)
- [17] M. Hoshino *et al.*: *J. Synchrotron Rad.* **21** (2014) 1347.
- [18] T. Tsukube *et al.*: *Gen. Thorac. Cardiovasc. Surg.* (2015) - in press.

Also read Pearson *et al.* in this volume.

Crystal structure of the LH1-RC complex in bacterial photosynthesis

Carbon, which is one of the most important elements for life, is taken up into biological system by photosynthesis. Purple bacteria have a simpler photosynthetic system than plants: the chromatophore vesicle of photosynthetic bacteria contains several protein components such as core antenna (LH1), peripheral antenna (LH2), reaction center (RC) and the high-potential iron-sulfur protein (HiPIP) (Fig. 1). LH1 and RC form a supramolecular complex (LH1-RC) to utilize sunlight energy in a highly efficient manner. Therefore, it is indispensable to know the atomic structure of the LH1-RC, including the arrangement of cofactors, to understand the mechanism of energy and electron transfer. However, only low-resolution structures of LH1-RC have been hitherto reported [1,2].

High quality crystals were obtained for the LH1-RC complex purified from a thermophilic bacterium, *Thermochromatium tepidum* (ttLH1-RC). The diffraction data were collected at beamlines BL41XU and BL44XU of SPring-8 and BL1A, BL17A, and NE3A of the Photon Factory. The phases were calculated with the multiple isomorphous replacement method with anomalous scattering (MIRAS) assisted by the molecular replacement (MR) method. Correction of diffraction anisotropy and multi-crystal averaging of the electron densities significantly improved electron density maps. The structures of LH1-RC in the two crystal forms ($P2_1$ and $C2$) were refined at 3.0 Å resolution [3].

The ttLH1-RC complex is composed of cytochrome, L, M and H subunits for RC, $\alpha_{16}\beta_{16}$ subunits for LH1, and ~80 cofactors. The RC complex is completely

surrounded by 16 $\alpha\beta$ -heterodimers that form an elliptical ring (Fig. 2(a)). The distances of its major and minor axes are 82 Å and 73 Å for the inner ring and 105 Å and 96 Å for the outer ring, respectively. The RC portion in the ttLH1-RC complex is almost identical to its isolated RC complex [4], but the ubiquinone molecule is bound to the binding site of RC in the ttLH1-RC complex (Fig. 2(b)). Each $\alpha\beta$ -heterodimer contains two bacteriochlorophyll (BChl), one spirilloxanthin (Spx), and one calcium ion. The two BChl molecules are bound to α -His36 and β -His36 in LH1 (Fig. 3(a)). BChl of the α -subunit forms a hydrogen bond with α -Trp46, whereas that of the β -subunit forms with β -Trp45. In addition, this BChl interacts with α -Gln28 and β -Trp28. BChl's bound to the α_1 , β_1 , α_9 , and β_9 subunits are almost parallel to the special pair BChl's (Fig. 2(a)). These parallel BChl's may play an important role in the efficient transfer of the excitation energy from LH1 to the special pair.

It has been revealed that the binding of calcium ions regulates the thermostability of the ttLH1-RC complex. In addition, an absorption maximum at 915 nm (Q_y transition of BChls in LH1), which is approximately 30 nm red-shifted from that of other purple bacteria, is also resulted by calcium ions. In the ttLH1-RC structure, each calcium ion in LH1 is coordinated by five oxygen atoms from α -Trp46, α -Asp49, α -Asn50, and the C-terminal carboxyl group of β -Leu46 in the adjacent subunit (Fig. 3(b)). The magnesium-magnesium distance of two BChl's is shortest in the bacterial light-harvesting antennas.

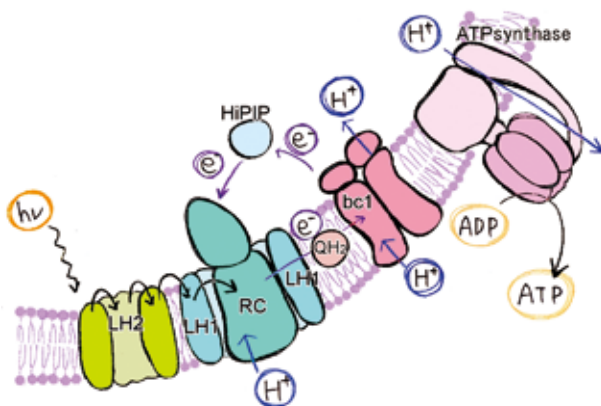


Fig. 1. Schematic representation of the chromatophore vesicle in photosynthetic purple bacteria. Only a part of the vesicle is shown. Vesicle contains photosynthetic proteins such as LH1-RC, LH2, cytochrome bc_1 complex (bc_1), ATP synthase and HiPIP. Reduced ubiquinone molecule (QH_2) is transferred from RC to bc_1 beyond the LH1 ring.

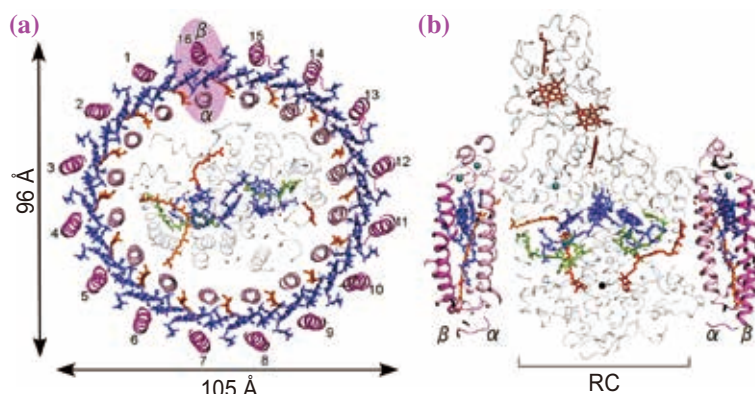


Fig. 2. Overall structure of ttLH1-RC. (a) Top view from the periplasmic side. The α and β -apoproteins in the LH1 ring are represented as light pink and pink ribbons, respectively. The subunits in RC are shown in grey. In addition, cofactors are represented in following colors: hemes, brown; calcium ions, green; BChl's, violet; bacteriopheophytin, yellow green; Spx, orange; quinones, red; and non-heme iron, black. A single $\alpha\beta$ -heterodimer is shaded in pink. (b) Side view of (a) rotated by 90° around the horizontal axis.

These observations indicate that the tight association of the α - and β -apoproteins mediated by the calcium ions has a critical contribution to thermostability and the large red-shift.

The LH1 ring has channels at the interface between adjacent $\alpha\beta$ -heterodimers. The channels and the ubiquinone binding site in RC are almost on the same level in the transmembrane region (Fig. 3(c)). The size of the channels is comparable to

that of the benzoquinone head group of ubiquinone. Therefore, the channels can play a role of passages for ubiquinone shuttling through the closed LH1 ring.

The structural information of ttLH1-RC enables us to understand the molecular mechanism of the excited energy transfer from LH1 to RC and the ubiquinone shuttling. In addition, the calcium-binding manner indicates the structural basis for the unique properties of ttLH1-RC.

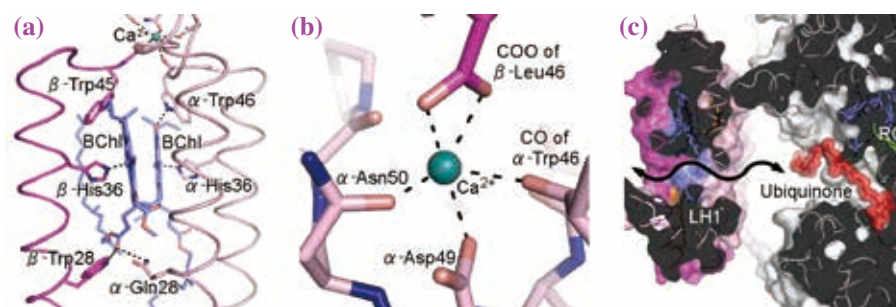


Fig. 3. Detailed views of ttLH1-RC. (a) Interactions around the BChl molecules in the LH1 ring. (b) Calcium binding site in the LH1 ring. (c) Cross section of ttLH1-RC at the ubiquinone transfer pathway.

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References

- [1] A.W. Roszak *et al.*: Science **302** (2003) 1969.
- [2] P. Qian *et al.*: Biochemistry **52** (2013) 7575.
- [3] S. Niwa L.J. Yu, K. Takeda, Y. Hirano, T. Kawakami, Z.Y. Wang-Otomo and K. Miki: Nature **508** (2014) 228.
- [4] T. Nogi *et al.*: Proc. Natl. Acad. Sci. USA **97** (2000) 13561.

Crystal structure of an associated form of NADPH-cytochrome P450 reductase with heme oxygenase

NADPH-cytochrome P450 reductase (CPR) supplies electrons to various heme proteins, including microsomal cytochrome P450s and heme oxygenase (HO). Thus, CPR is fundamentally important for metabolism of xenobiotics, steroids, and heme. In fact, some mutations of CPR impair biosynthesis of steroid hormones, which may be a possible etiology of Antley-Bixler syndrome. CPR contains two redox coenzymes (FMN and FAD). Electrons from NADPH flow first to FAD, then to FMN, and finally to heme in the redox partner. CPR has at least two distinct conformations (open and closed). In the closed conformation [1], the FMN-binding site is covered by FAD-binding domain, and as a result, FMN is not exposed to the surface. However, rapid electron transfer from NADPH to FMN could take place in this conformation because FMN and FAD are in close proximity. In the open conformation [2], FMN is exposed to the surface. Therefore, this conformation should be favorable for interactions with a redox partner. To date, structures of CPR in a complex with its redox partner have yet to be determined.

HO catalyzes heme degradation, utilizing three oxygen molecules and seven electrons to produce biliverdin, ferrous ion, and carbon monoxide (Fig. 1).

The seven electrons required for the HO reaction are provided by CPR. Both CPR and HO are localized in the microsomal membrane *in vivo*, but *in vitro*, a solubilized rat CPR (sCPR), in which the membrane-bound N-terminal segment has been truncated, can support heme degradation catalyzed by solubilized rat HO-1 (sHO-1), with a truncated membrane-bound C-terminal segment. Extensive crystallographic, spectroscopic, and biochemical studies of sHO-1 have revealed the unique mechanism of the HO reaction [3,4]; however, how the reducing equivalents are transferred from CPR to HO will remain unclear until the tertiary structures of the associated forms of CPR with HO are determined.

Although the tertiary structure of the associated form of CPR with HO is interesting, it is difficult to crystallize due to its instability. To overcome this problem, we attempted to prepare a stable associated form using mutations of sCPR. A mutated sCPR, designated Δ TGEE, can keep stable open conformations [2] and associate with heme-bound sHO-1 (heme-sHO-1). Δ TGEE can support the HO reaction, although its efficiency is extremely limited.

Furthermore, we have determined the crystal structure of Δ TGEE in a complex with heme-sHO-1

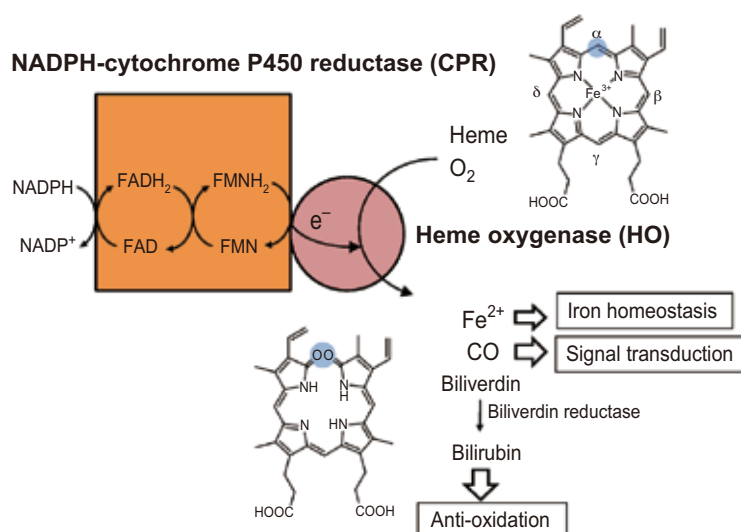


Fig. 1. Physiological functions of heme metabolism. HO catalyzes heme degradation utilizing three oxygen molecules and seven electrons to produce biliverdin, ferrous ion, and carbon monoxide. Ferrous ions produced by the HO reaction are pivotal for iron homeostasis because ~95% of the daily iron requirement is supplied by recycling the iron released by this reaction. In addition, bilirubin produced from biliverdin by biliverdin reductase plays a major role as an anti-oxidant at its physiological concentration. Carbon monoxide is suggested to be involved in several cell signaling processes, such as anti-inflammation, anti-apoptosis, and vascular constriction and dilation.

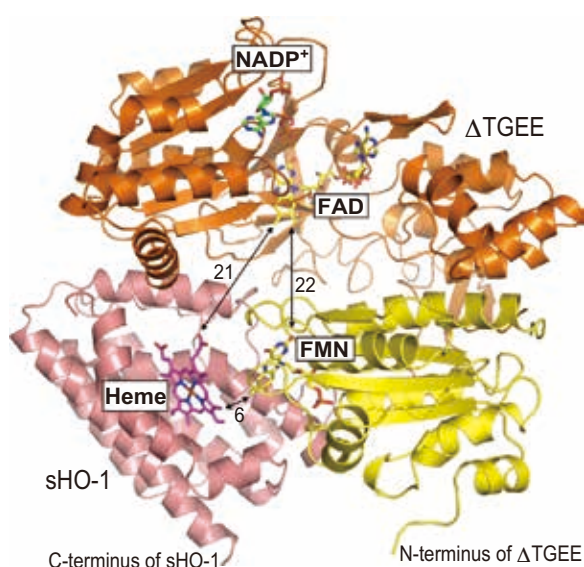


Fig. 2. Crystal structure of Δ TGEE-heme-sHO-1 complex. Orange and yellow ribbon diagrams show FAD and FMN binding domains of Δ TGEE, respectively. Pink ribbon diagram shows sHO-1. NADP⁺, FAD, FMN, and heme are shown with stick models. Numerals stand for various distances in Angstrom.

at 4.3 Å resolution by molecular replacement using synchrotron radiation data collected at beamline BL44XU [5]. Δ TGEE assumes the open conformation in the complex structure. The N-terminal side of

Δ TGEE and the C-terminal side of heme-sHO-1 face the same direction, indicating that the microsomal membrane into which these terminals are embedded *in vivo* does not interfere with the association of CPR and HO (Fig. 2). X-ray scattering and cross-linking analyses indicate that the structure of Δ TGEE and heme-sHO-1 complex is almost identical to that of sCPR and heme-sHO-1 complex.

The short distance (6 Å) between heme and FMN in the Δ TGEE and heme-sHO-1 complex implies that direct electron transfer from FMN to heme is plausible. On the other hand, FAD is far from FMN and heme (~20 Å), suggesting that electron transfer from FAD to FMN is difficult if CPR keeps the open conformation. This would explain the extremely limited heme degradation efficiency of the Δ TGEE and heme-sHO-1 complex. Based on the structure, we propose an electron transfer mechanism from CPR to HO that accompanies the dynamic conformational changes of CPR (Fig. 3). CPR is in a dynamic equilibrium between the open and closed conformations. When NADPH binds to CPR, FMN is readily reduced, and then CPR changes to the open conformation. The open CPR can bind to heme-HO-1, which transfers an electron from FMN to heme. FMN is subsequently re-oxidized and CPR changes to the closed conformation to release HO-1. The “closed-open transition” of CPR should be indispensable for smooth electron transfer from FAD to FMN and the dissociation of HO from CPR.

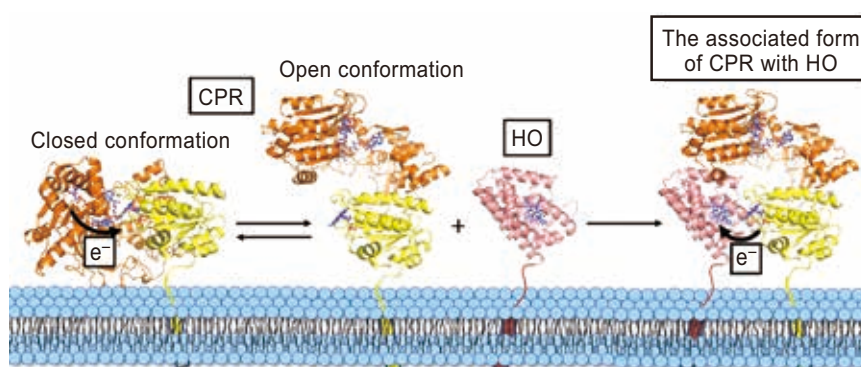


Fig. 3. Mechanism for electron transfer from CPR to heme-HO-1 that accompanies the dynamic conformational changes of CPR.

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References

- [1] M. Wang *et al.*: Proc. Natl. Acad. Sci. USA **94** (1997) 8411.
- [2] D. Hamdane *et al.*: J. Biol. Chem. **284** (2009) 11374.
- [3] G. Kikuchi *et al.*: Biochem. Biophys. Res. Commun. **338** (2005) 558.
- [4] T. Matsui *et al.*: Acc. Chem. Res. **43** (2010) 240.
- [5] M. Sugishima, H. Sato, Y. Higashimoto, J. Harada, K. Wada, K. Fukuyama, M. Noguchi: Proc. Natl. Acad. Sci. USA **111** (2014) 2524.

Crystal structure of voltage-gated proton channel

Voltage-gated proton channel, VSOP (or Hv1), plays dual roles as a voltage sensing and proton permeation. VSOP was first suggested to be involved in bioluminescence in dinoflagellates, and was identified in *Mus musculus* and *Homo sapiens* in 2006 [1,2]. VSOP is required for high-level superoxide production by phagocytes through its tight functional coupling with NADPH oxidase to eliminate pathogens. Hv1 is expressed in human sperm, where its suggested role is to regulate motility through activating alkalization-activated calcium channels. The activities of Hv1 also have pathological implications such as exacerbation of ischemic brain damage and progression of cancer.

VSOP only has a voltage-sensor domain (VSD) that consists of four-transmembrane helices, which are similar to the VSD of typical voltage-gated ion channels such as voltage-gated sodium (Nav), potassium (Kv), or calcium (Cav) channels and voltage sensor-containing phosphatases [3]. In biological membranes, VSOP is expressed as a dimer, which depends on the dimerization of the cytoplasmic coiled-coil. The fourth-transmembrane helix (S4), which has periodically aligned positive charge residues such as arginines, is known to change its orientation relative to other helices in VSD upon a membrane potential change (Fig. 1(a)). Thus, VSD is an individual module-like domain; not only can it convert the membrane potential changes into several biological signals by exchanging a connected domain such as ion pore and phosphatase, but it can also permeate protons as a voltage sensor in the case of VSOP (Fig. 1(b)).

However, the mechanism of voltage sensing is unknown because all crystal structures of VSDs have been determined as activated structures since almost all typical voltage gated ion channels show the activated state in cancelled membrane potential (0 mV), which is equal to the solubilization-state using detergent for protein preparation. Although the mechanism seems to depend on membrane potential, the discussion of the resting structure is just conjecture. The predicted resting structure via computational techniques has not been completely explained. On the other hand, VSOP would be an agreeable model protein to understand the mechanism of voltage sensor, because the solubilized VSOP is considered to be closed-form and the promoter also shows proton channel activity. Moreover, VSOP activation is inhibited by Zn^{2+} , which is consistent with the property where almost all voltage sensors are inhibited by bivalent metal ions [4]. Thus, VSOP is not only the most compact voltage sensor protein but also

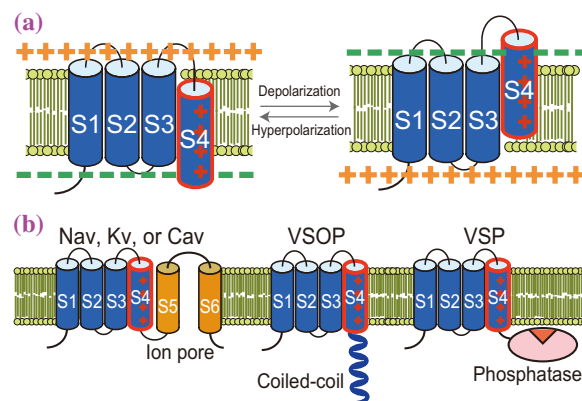


Fig. 1. S4 motion of VSD and VSD protein family. **(a)** Schematic illustration of the S4 motion model. Electrophysiological studies have shown that S4 moves upward and downward relative to other helices (S1 – S3) upon depolarization and hyperpolarization, respectively. **(b)** Voltage sensor protein superfamily contains a canonical voltage-gated ion channel, VSOP, and VSP. In particular, the property of S4's periodical aligned positively charged residues is highly conserved in this superfamily.

has a voltage sensing characteristic. In this report, we explain the crystal structure of the resting state VSOP in the presence of Zn^{2+} [5].

The crystallization construct (mHv1cc) is a chimeric channel, in which the cytoplasmic coiled-coil (Val216 – Asn269) and S2–S3 half intracellular-side (Glu149 – Phe171) are replaced with the GCN4 leucine zipper from *S. cerevisiae* (Arg249 – Arg281) and the intracellular portion of Ci–VSP (Asp164 – Leu188), respectively. We obtained mHv1cc crystals by vapor diffusion method. All reflection data sets were collected at 90 K on **BL44XU** beamline. The initial electron density map was obtained by the multiple anomalous diffraction method. The assignments of S2 and the S4 were performed using the anomalous signals of three selenomethionines (SeMet) in the S2 and the S4 transmembrane helices. Moreover, the S1 and S3 registers were assigned using L107M/L118M and L182M mutants substituted by SeMet. As a result, we determined the crystal structure of mHv1cc at a 3.45-Å resolution in which the R and R_{free} values were refined 34.1% and 35.7%, respectively. The crystal structure of mHv1cc shows that the entire shape looks like “closed Wagasa (traditional Japanese umbrella)” (Fig. 2(a)). We also identified Zn^{2+} in mHv1cc using two wavelength data collected below and above the absorption edge of zinc (1.290 Å and 1.275 Å). Two histidine residues, His136 and His189 of mHv1cc, which are known to be critical for Zn^{2+} binding in hHv1, exist at positions that

should coordinate with Zn^{2+} . His189 was disordered and could not be modeled in the electron density map, but it also would be positioned near Zn^{2+} . In addition, we found two negatively charged residues, Glu115 and Asp119, near Zn^{2+} (Fig. 2(b)). Thus, four residues likely contribute to Zn^{2+} binding in the non-activated state or closed form because Zn^{2+} specifically inhibits the activities of VSOP.

Until now, all of these VSD crystal structures were considered to be in the activated state as evidenced by their biophysical and electrophysiological properties. The positive charged residues of S4 helices in Kv1.2–2.1 chimeric channel and other VSDs are located above a charge transfer center. In the case of mHv1cc, it seems that two of the sensor residues, Arg204 and Arg207, slide toward the inner membrane side relative to the conserved phenylalanine, Phe146, on S2 in a charge transfer center (Fig. 3(a)). This along with the Zn^{2+} -binding and the location of S4 indicates that this structure is a resting-state of the mHv1cc VSD structure.

The mHv1cc structure has two hydrophobic layers (Fig. 3(b)). The lower layer at the cytoplasmic side (HL_{in}) includes Phe146 and Phe178, while the upper layer is on the extracellular-side (HL_{ex}), and consists of four highly conserved hydrophobic residues from four helices (Val112(S1), Leu143(S2), Leu185(S3) and Leu197(S4)). HL_{ex} and HL_{in} may also prevent the penetration of water molecules, which can act as proton carriers. In our mHv1cc structure, Asp108, which is critical for proton selective permeation, seems to be in the hydrophobic layer. However, recent PEGylation experiments to detect the accessibility of maleimide-reagents suggest that Asp108 faces an aqueous vestibule [5]. The two hydrophobic layers probably

play distinct roles in regulating the proton conduction pathway.

In conclusion, we not only determined the first VSOP crystal structure, but we also observed the likely resting state structure of VSD. Future elucidation of the crystal structures of VSOP at higher resolutions and those of other gating states should unravel the detailed operating mechanisms of VSOP and the general principles of VSD, which are common among voltage-gated ion channels and voltage-sensing phosphatases upon membrane potential changes.

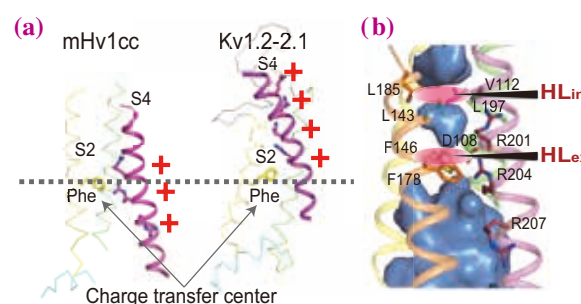


Fig. 3. Comparison of the S4 positional relationship corresponding to the charge transfer center between the mHv1cc and Kv1.2–2.1 (PDB ID: 2R9R) chimeric channel and intermolecular double hydrophobic barriers of mHv1cc. **(a)** Yellow stick model depicts these highly conserved hydrophobic residues (phenylalanine) and the charge transfer center on S2. **(b)** Double hydrophobic layers consist of the hydrophobic residues, which are shown by a stick model. These water-accessible regions are drawn using a Connolly surface (probe radius = 1.4 Å, blue surface).

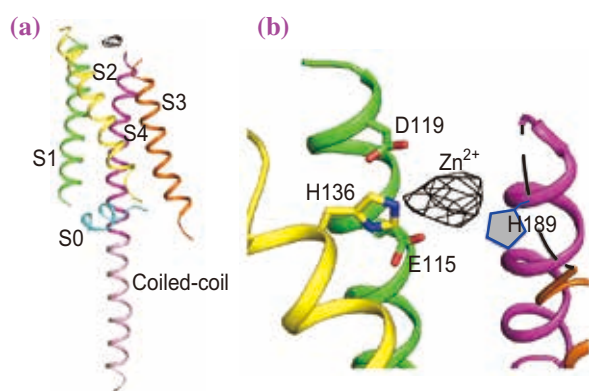


Fig. 2. Crystal structure and Zn^{2+} binding site of mHv1cc. **(a)** Four transmembrane helices are distinguished by color: S1 (green), S2 (yellow), S3 (orange), and S4 (magenta) connected to cytoplasmic coiled-coil region (pink). N-terminal cytoplasmic helix (S0) is cyan. **(b)** Black mesh shows the Zn^{2+} -anomalous difference map contoured at the 5.0σ level.

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References

- [1] M. Sasaki *et al.*: Science **312** (2006) 589.
- [2] I.S. Ramsey *et al.*: Nature **440** (2006) 1213.
- [3] Y. Murata *et al.*: Nature **435** (2005) 1239.
- [4] T. Kurokawa *et al.*: Biochem. Biophys. Acta. **1838** (2014) 382.
- [5] K. Takeshita, S. Sakata, E. Yamashita, Y. Fujiwara, A. Kawanabe, T. Kurokawa, Y. Okochi, M. Matsuda, H. Narita, Y. Okamura and A. Nakagawa: Nat. Struct. Mol. Biol. **21** (2014) 352.

Crystal structure of heterotetrameric NMDA receptor reveals insights into subunit arrangement and function

N-methyl-D-aspartate (NMDA) receptors mediate fast neurotransmission that is pivotal in brain development and basic function including learning and memory formation. They are ligand-gated ion channels that open upon binding of neurotransmitter, glutamate, along with a co-agonist glycine or D-serine (Fig. 1). Dysfunction of NMDA receptors is implicated in neurological disorders and diseases including depression, schizophrenia, Alzheimer's disease, Parkinson's disease, and ischemic injuries associated with stroke. There has been a great enthusiasm toward understanding the structure and function of NMDA receptors at the molecular level owing to their importance in basic neuroscience as well as disorders and diseases. Using BL41XU beamline at SPring8 and ID23 beamline at Advanced Photon Source at the Argonne National Laboratory, we obtained the first crystal structure of heterotetrameric NMDA receptors containing an amino terminal domain (ATD), a ligand-binding domain (LBD), and a transmembrane domain (TMD) [1].

Structural studies of NMDA receptors has been considered challenging since they are obligatory heterotetramers composed of large transmembrane subunit proteins, namely GluN1 and GluN2 subunits. Toward obtaining the crystal structure, we extensively tested various protein constructs and expression methods and found that the best yield of properly assembled heterotetrameric GluN1a/GluN2B NMDA receptors can be obtained by truncating the carboxy terminal domain (CTD) (hence called GluN1a/2B_{cryst}) and by using a combination of *Sf9* insect cells with a

recombinant baculovirus under the Hsp70 promoter from *Drosophila melanogaster*.

The GluN1a/2B_{cryst} construct was crystallized in the presence of the GluN1 agonist, glycine, the GluN2 agonist, glutamate, and an ATD-binding allosteric inhibitor, ifenprodil. The structure was initially solved at 5.7 Å by molecular replacement using the structures of the ATD [2] and LBD [3] as search probes. To improve the diffraction power of these crystals, we stabilized the heterotetrameric subunit interactions by forming inter-subunit disulfide cross-links at the extracellular and juxtamembrane regions based on the 5.7 Å structure above. This construct manipulations improved the diffraction limit to beyond 4 Å, which resulted in electron density sufficient to build most of the GluN1a/2B NMDA receptor including the entire extracellular domains, TMD, linkers between ATD and LBD and between LBD and TMD.

The GluN1a/GluN2B NMDA receptor bound to glycine, L-glutamate, and ifenprodil is shaped like a "hot-air balloon" where a balloon and a basket correspond to the entire extracellular domains and the TMD, respectively. The structure has a clear boundary between the layers of LBD and TMD while ATD and LBD appear as a single unit. Both ATD and LBD form two GluN1a-GluN2B heterodimers that are assembled as GluN1-GluN2-GluN1-GluN2 (1-2-1-2) heterotetramer where the two-fold symmetry axis runs across the center of the molecule through the interface between the two GluN2B ATDs to the middle of the ion channel pore (Fig. 2). The overall shape of GluN1a/GluN2B NMDA receptor is highly distinct from that of homotetrameric GluA2 AMPA receptor, another member of the iGluR family, which has the overall "Y" shape [4]. This difference in the overall architecture is attributed to the fact that ATD and LBD pack tightly through numbers of well-defined inter-domain interfaces in GluN1a/GluN2B NMDA receptors whereas ATD and LBD interact minimally in GluA2 AMPA receptors. This surprisingly "compact" architecture of GluN1a/GluN2B NMDA receptor stems from the inter-subunit and inter-domain interactions over large surface areas that are unique to NMDA receptors.

The TMD of GluN1a/GluN2B NMDA receptor forms the heterotetrameric ion channel with pseudo four-fold symmetry. The ion channel of GluN1a/GluN2B NMDA receptor is similar to the closed state of GluA2 AMPA receptors and KcsA bacterial potassium channels. In this plausible allosterically inhibited state, the ion channel is closed to the similar extent to the

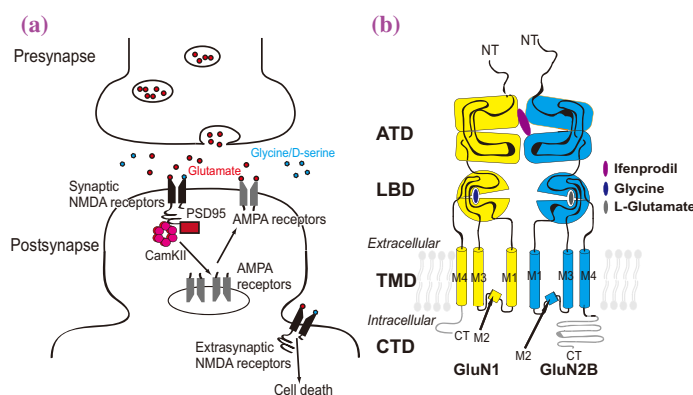


Fig. 1. NMDA receptors (a) NMDA receptors are critical for neuronal signaling involved in brain development and function. Binding of glutamate and glycine or D-serine opens NMDA receptor ion channels and facilitate high calcium influx. (b) NMDA receptors are heterotetrameric ion channels composed of two subunits, GluN1 and GluN2 and in some cases GluN3. Each subunit is composed of ATD, LBD, TMD, and CTD.

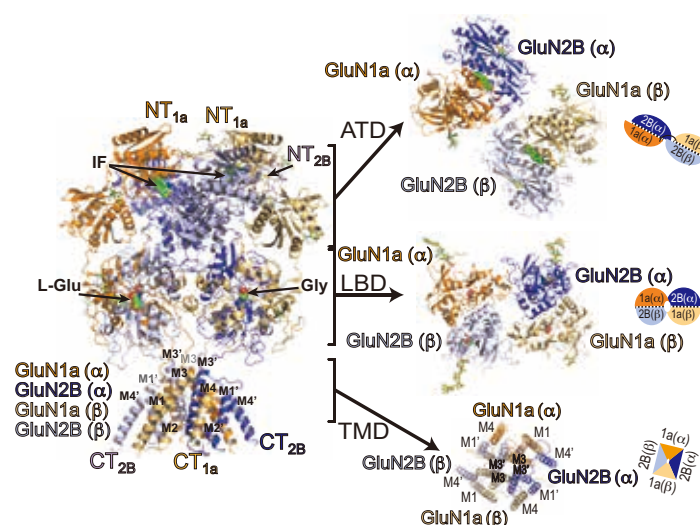


Fig. 2. The first crystal structure of heterotetrameric NMDA receptors. We crystallized the GluN1/GluN2B NMDA receptors without CTD. The heterotetramers are formed as a dimer of heterodimer in the GluN1-GluN2B-GluN1-GluN2B arrangement in each domain layer. Interestingly, dimer pairs are swapped between ATD and LBD.

closed channels of AMPA receptor and KcsA. One of the key functions of NMDA receptors is their high permeation of calcium ions, which plays the major role in neuronal plasticity as well as excitotoxicity. The crystal structure complexed with holmium, a lanthanide known to recognize calcium binding sites in many biological macromolecules, shows the binding in between the LBD-TMD linkers of GluN1 right above the center of the ion channel (Fig. 3). A set of acidic residues in GluN1 (DRPEER motif) located in this region has been shown previously to be a critical part of high calcium flux characteristic of NMDA receptors [5]. Thus, the lanthanide binding site along with the previous electrophysiological study further confirms the

physiological relevance of the current crystal structure. However, despite extensive efforts, the regions of TMD that determines voltage-dependent Mg^{2+} block and Ca^{2+} permeation was not clearly resolved in this crystal structure. Structure-based understanding of ion selectivity and flux regulation is thus a question that remains to be addressed.

The crystal structure of GluN1a-GluN2B NMDA receptors marks the first crystal structure of a heteromeric ion channel. The structure will serve as a template for designing experiments that further address complex function of NMDA receptors. Finally, the defined subunit interfaces should serve as an invaluable blueprint for design of therapeutic compounds.

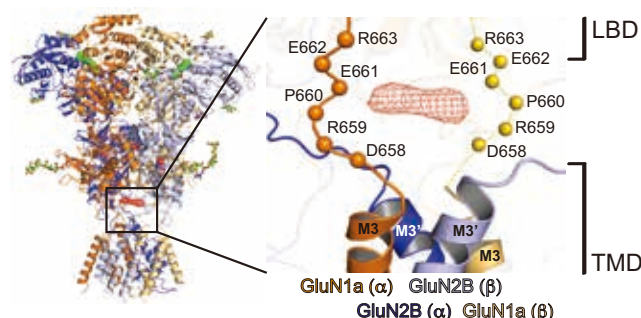


Fig. 3. Plausible calcium binding site at the juxtamembrane site. Here anomalous difference Fourier map obtained from diffraction data of crystals soaked against holmium is shown. Holmium has been previously shown to bind calcium binding site.

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References

- [1] E. Karakas and H. Furukawa: *Science* **344** (2014) 992.
- [2] E. Karakas *et al.*: *Nature* **475** (2011) 249.
- [3] A. Jespersen *et al.*: *Neuron* **81** (2014) 366.
- [4] A.I. Sobolevsky *et al.*: *Nature* **462** (2009) 745.
- [5] J. Watanabe *et al.*: *J. Neurosci.* **22** (2002) 10209.

Agonist-bound structure of purinergic receptor P2Y₁₂

GPCRs activate associated intracellular signal transduction pathways after sensing their endogenous extracellular ligands. Rhodopsin, β_1 and β_2 adrenergic receptors, A_{2A} adenosine receptor and M2 muscarinic receptor have both antagonist- and agonist-bound structures now. Each of these five receptors, however, belongs to the α -group of class A GPCRs [1]. Here, we describe a 2.5 Å structure of human P2Y₁₂R bound to the full agonist 2MeSADP, and a 3.1 Å structure of P2Y₁₂R bound to a potential partial agonist 2MeSATP. Together with the 2.7 Å structure of P2Y₁₂R bound to the antagonist AZD1283 reported in the accompanying paper [2], this allows the first crystallographic assessment of a receptor with both agonist- and antagonist-bound structures in the δ -group of class A GPCRs.

All three P2Y₁₂R structures were determined using the same thermostabilizing construct. Protein sample of P2Y₁₂R was reconstituted into lipidic cubic phase (LCP) for crystallization trials. Their crystals (80×50×5 μm^3 for 2MeSADP-P2Y₁₂R and 50×50×5 μm^3 for 2MeSATP-P2Y₁₂R) diffracted to 2.5 Å and 3.1 Å at maximum on beamline **BL41XU**, respectively. Diffraction data sets were collected by exposing crystals with a 10 μm minibeam for 1 second and 1° oscillation per frame. The structures were solved by molecular replacement and refined in Refmac5 and Buster.

It is observed that the receptor structure of 2MeSADP and 2MeSATP bound complexes are very similar (Fig. 1(a)), indicating that the ligand 2MeSATP could potentially induce similar conformation change of P2Y₁₂R and activate the receptor to some extent. Thus the tri-phosphate ligands and their derivatives could potentially be partial agonists, instead of antagonists. This hypothesis is further supported by the pharmacological data of AR-C66096, and our finding ends the debate of the pharmacological properties of ATP and 2MeSATP in P2Y₁₂R signaling. In the crystal structure of antagonist bound P2Y₁₂R structure, there is a usual straight conformation and tilted orientation of helix V observed due to the lack of a conserved proline in this helix, which is further confirmed in the agonist bound structures. Considering the facts that the orientations of the fusion protein are different in all three structures, the straight confirmation of helix V is likely a genuine structural feature of the P2Y₁₂R [2].

The agonist bound and antagonist bound P2Y₁₂R structures show remarkable differences at the extracellular end. The inward shift of helices VI and VII in the 2MeSADP complex is striking and the N terminus

is moved towards the axis of the helical bundle as well (Fig. 1(b)). The inward movement of these helices is consistent with previous structures, while the scale could not be previously predicted. In the previous structure of PAR1, the authors ran a long simulation try to reveal how the ligand vorapaxar gains access into the binding cavity of the receptor, but without any success [3]. This could be now explained by the very high plasticity of the extracellular region, and this could be a common structural feature of some GPCRs. Interestingly, the intracellular changes of P2Y₁₂R are less prominent than those at the extracellular side (Fig. 1(b)). The P2Y₁₂R-2MeSADP structure possibly represents an “agonist-bound inactive state” with respect to the intracellular region, similar to the one observed in agonist-bound β_1 AR and β_2 AR without G-protein or a G-protein mimic stabilizing their active state [4,5].

The 2MeSADP-binding pocket consists of residues from helices III, IV, V, VI and VII as in the P2Y₁₂R-AZD1283 structure [2], but also extensively involves ECL2 and the N-terminus. The adenine group of 2MeSADP forms a similar π - π interaction with the Y105 side chain. The 2-thioether inserts into a hydrophobic pocket and serves as an anchor to maintain the adenine core and the ribose ring in an optimal orientation (Fig. 2). Thus, 2MeSADP binds with greater complementarity, which explains the higher affinity of this ligand compared to ADP.

In the antagonist bound P2Y₁₂R structure, the conserved disulfide bond between TM3 and ECL2 is very flexible and is not built in the structure, while it is

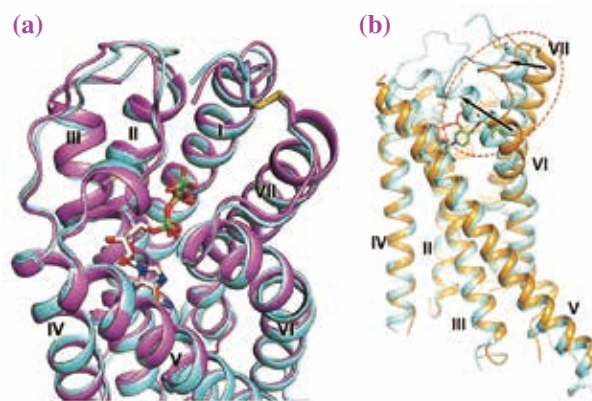


Fig. 1. Structure of the P2Y₁₂R-2MeSADP, P2Y₁₂R-2MeSATP and P2Y₁₂R-AZD1283 complexes. (a) Comparison of P2Y₁₂R-2MeSADP (cyan) with P2Y₁₂R-2MeSATP (violet) complex. 2MeSATP is colored in green. (b) Comparison of P2Y₁₂R-2MeSADP with P2Y₁₂R-AZD1283 complex (receptor: light orange cartoon and ligand: sticks of green carbons).

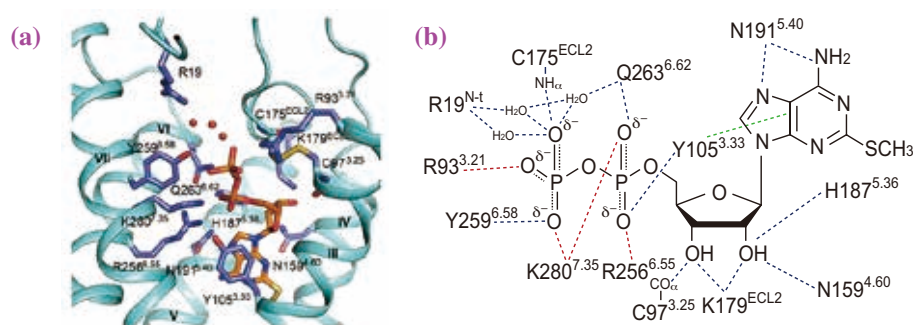


Fig. 2. P2Y₁₂R ligand binding pocket for 2MeSADP. **(a)** The ligand 2MeSADP (orange carbons) and receptor residues (slate carbons) involved in ligand binding are shown in stick representation. The water molecules interacting with 2MeSADP are shown as red spheres. **(b)** Hydrogen bonds are displayed as blue dashed lines and the salt bridges as red dashed lines. The π - π interaction between 2MeSADP and Y105 is indicated as green dashed lines. The NH α and CO α indicate the main chain amine and carbonyl groups of the corresponding residue.

observed in two agonist-bound structures. However, to form this disulfide, the C α of C97 is required to rotate along the helical path by over 60° and the extracellular tip of helix V is also shifted ~2 Å towards the helical bundle as compared with the AZD1283 complex. This difference indicates a dynamic disulfide of P2Y₁₂R, and different state of this disulfide is required by different type of ligand binding.

The mechanism of agonist and antagonist interactions with P2Y₁₂R can be schematically illustrated in Fig. 3. Agonist access to the binding pocket of apo P2Y₁₂R would require plasticity of this extracellular region caused by charge repulsion from

the side chains of ~8 arginine and lysine around the pocket. Binding of a nucleotide agonist like 2MeSADP electrostatic attraction between charged phosphates and positively charged residues, and inward position of helices VI and VII and the electrostatic “lid” are stabilized. The additional phosphate group of 2MeSATP is also accommodated by a similar conformation of the lid, though distinct interactions may still impact the 2MeSATP binding and signaling profile. In stark contrast, binding of non-nucleotide antagonists like AZD1283 has an open extracellular side conformation with helices VI and VII away from the pocket and destabilizes the lid.

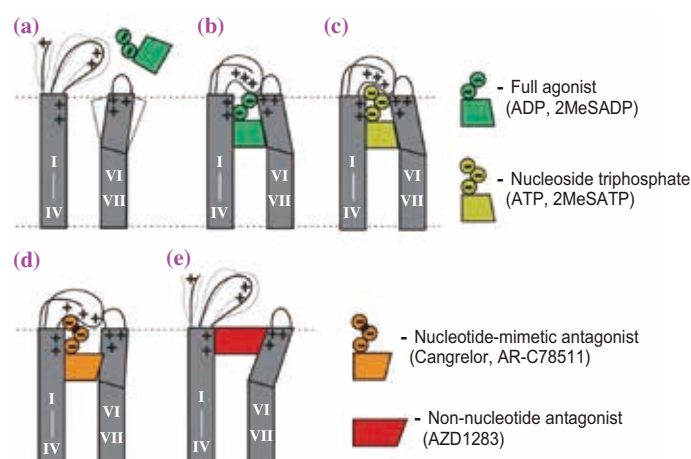


Fig. 3. Schematic illustration of conformational changes in P2Y₁₂R extracellular region.

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References

- [1] R. Fredriksson *et al.*: Mol. Pharmacol. **63** (2003) 1256.
- [2] K. Zhang, J. Zhang, Z.G. Gao, D. Zhang, L. Zhu, G.W. Han, S.M. Moss, S. Paoletta, E. Kiselev, W. Lu, G. Fenalti, W. Zhang, C.E. Müller, H. Yang, H. Jiang, V. Cherezov, V. Katritch, K.A. Jacobson, R.C. Stevens, B. Wu and Q. Zhao: Nature **509** (2014) 115.
- [3] C. Zhang *et al.*: Nature **492** (2012) 387.
- [4] T. Warne *et al.*: Nature **469** (2011) 241.
- [5] D.M. Rosenbaum *et al.*: Nature **469** (2011) 236.

Crystal structure of YidC reveals a mechanism of Sec-independent membrane protein

The plasma membrane, which is mainly composed of phospholipids and membrane proteins, works as a boundary between the inside and outside of living cells. A variety of membrane protein functions in processes that take place within and across the membrane, such as energy generation, signal transduction, and ion transport. These membrane proteins are synthesized in the cytoplasm and are then inserted into the membrane by protein machineries. The YidC/Oxa1/Alb3 family proteins play key roles in protein insertion into bacterial, mitochondrial and thylakoidal membranes, respectively.

In the bacterial inner membrane, YidC inserts proteins into the membrane on its own, facilitates proper folding of membrane proteins inserted by a protein-conducting channel, the SecYEG translocon, and is essential for cell viability [1,2] (Fig. 1). Recent studies have revealed that Sec translocon possesses an aqueous channel for translocation of hydrophilic peptides across the membrane as well as membrane protein insertion. In contrast, the lack of high-resolution structures of YidC hampers the understanding of the molecular mechanisms for membrane protein insertion mediated by YidC. Here, we determine the crystal structure of YidC from *Bacillus halodurans* at 2.4 Å resolution by the multiwavelength anomalous diffraction method using a mercury-derivatized crystal [3,4]. All diffraction data were collected at beamline BL32XU.

The YidC structure consists of an N-terminal helix (EH1), two cytoplasmic helices (CH1 and CH2), and a core region composed of five transmembrane helices (TM1–5), which reveal a novel protein fold (Fig. 2(a)). The EH1, CH1, and CH2 helices lie parallel to the membrane surface. The amphipathic EH1 helix is partly embedded in the membrane, while the CH1 and CH2 helices are composed of mainly polar or charged residues that are fully exposed to

the cytoplasm. The TM1–5 helices form a hydrophilic groove in the cytoplasmic leaflet, which is open to both the membrane interior and the cytoplasm, but is closed on the extracellular side by the hydrophobic core of the protein (Fig. 2(b)). This groove contains many hydrophilic residues (Fig. 2(c)), generating a hydrophilic environment that spans the cytoplasmic leaflet of the membrane. In particular, the conserved arginine, Arg72, is present in the center of the groove, which results in a positive electrostatic potential on the groove surface (Fig. 2(b)). Molecular dynamic simulations show that the groove can accommodate about 20 water molecules, but the water molecules do not permeate the membrane during a 1-μs simulation. These observations suggest that YidC, unlike the Sec translocon, does not possess a pore for protein translocation.

To investigate the functional importance of the hydrophilic groove, we performed a genetic analysis of SpoIIIJ, the YidC orthologue in *Bacillus subtilis* using a β-galactosidase assay to monitor the membrane insertion of MifM [5], a substrate protein that is inserted in a SpoIIIJ-dependent manner. Substitution of the arginine, Arg73 in SpoIIIJ, with an alanine abolished MifM insertion activity, while substitution of other conserved glutamines in the groove with an alanine did not affect the activity. In contrast, a lysine substitution of the arginine retained its partial activity. These results suggested that a positively charged residue is important in the hydrophilic groove in order for YidC activity to act as an insertase. MifM is a single-spanning membrane protein, possessing three positively charged residues in the N-terminal extracellular regions. Next we mutated the positively charged residues to neutral residues and assessed the membrane insertion of the MifM mutants. The results showed that these mutations negatively

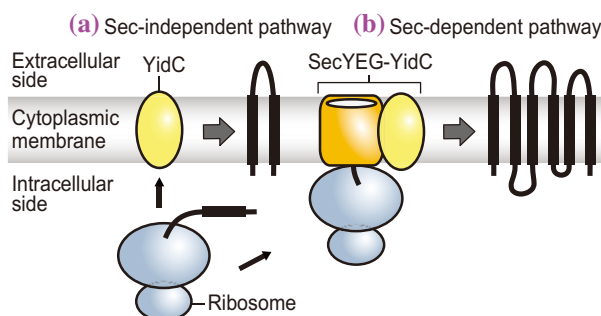


Fig. 1. YidC plays two different roles in membrane protein insertion. (a) YidC mediates membrane protein insertion on its own. (b) YidC works as a membrane chaperone in cooperation with Sec translocon to facilitate membrane protein folding and assembly.

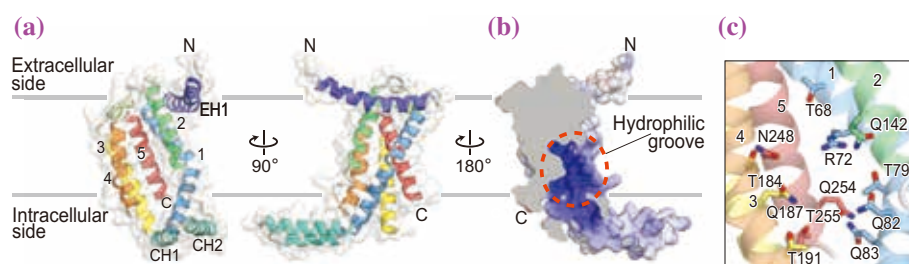


Fig. 2. Crystal structure of YidC. **(a)** Cartoon model of YidC, colored blue to red from the N to C terminus; with the surface model (gray) **(b)** Cross-sectional view of the hydrophilic groove. Surface is colored by the electrostatic potential, ranging from blue (+20 kT/e) to red (-20 kT/e). **(c)** Close-up view of the hydrophilic groove, showing the side chains of hydrophilic residues.

affected the membrane insertion efficiency of MifM, supporting the notion that the positive charge in the hydrophilic groove can attract the negative charge of the substrate protein. This assertion is also supported by *in vivo* site directed photo-crosslinking analysis, which shows direct interactions between the hydrophilic groove and MifM. Altogether, these results suggest that the hydrophilic groove plays a key role in membrane protein insertion as a substrate binding site.

On the basis of the crystal structure and the genetic and biochemical analyses, we propose the following model for membrane protein insertion mediated by YidC (**Fig. 3**). First, YidC interacts with the extracellular

region of the substrate using the hydrophilic groove, which transfers the extracellular region into the membrane. Then the extracellular region is released from the groove and fully translocated across the extracellular leaflet. Next the transmembrane region of the substrate is inserted into the membrane. It should be noted that membrane protein insertion could be promoted by various factors, such as the electrostatic forces by the membrane potential and the hydrophobic interaction between the transmembrane region and the membrane. Overall, our findings pave the way for further investigations into the molecular mechanism of YidC.

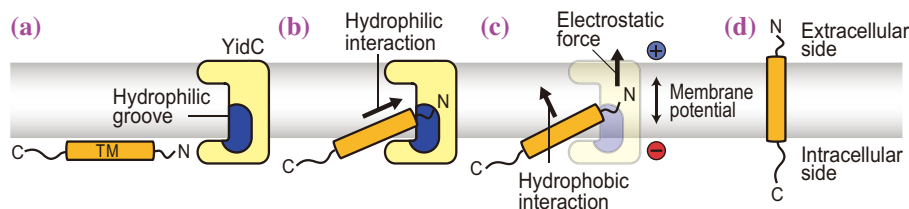


Fig. 3. Model for membrane protein insertion mediated by YidC. Schematic representation of membrane insertion of a single spanning protein that possesses an N-terminal extracellular tail, such as MifM. **(a, b)** Hydrophilic groove of YidC captures the N-terminal tail of substrate, which transfers the N-terminal tail into the membrane. **(c, d)** Subsequently, the N-terminal tail is translocated across the extracellular leaflet while the transmembrane region (TM) is released into the membrane, which could be facilitated by the hydrophobic interaction and the membrane potential.

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References

- [1] J.C. Samuelson *et al.*: Nature **406** (2000) 637.
- [2] R.E. Dalbey *et al.*: Biochim. Biophys. Acta **1843** (2014) 1489.
- [3] K. Kumazaki, S. Chiba, M. Takemoto, A. Furukawa, K.-I. Nishiyama, Y. Sugano, T. Mori, N. Dohmae, K. Hirata, Y. Nakada-Nakura, A. D. Maturana, Y. Tanaka, H. Mori, Y. Sugita, F. Arisaka, K. Ito, R. Ishitani, T. Tsukazaki and O. Nureki: Nature **509** (2014) 516.
- [4] K. Kumazaki, T. Tsukazaki, T. Nishizawa, Y. Tanaka, H.E. Kato, Y. Nakada-Nakura, K. Hirata, Y. Mori, H. Suga, N. Dohmae, R. Ishitani and O. Nureki: Acta Crystallogr. F **70** (2014) 1056.
- [5] S. Chiba *et al.*: EMBO J. **28** (2009) 3461.

Structure of the human DDB1–Cereblon – thalidomide complex

More than 50 years have passed since thalidomide was first prescribed as a sedative and antiemetic to provide effective relief from morning sickness during early pregnancy. Although the teratogenic effects associated with its use were soon discovered, more than 10,000 babies were born with severe birth defects such as phocomelia and amelia before thalidomide was withdrawn from the market. Pharmacological studies aimed at delineating the cause of thalidomide-induced teratogenicity led to the discovery of a number of unexpected pharmacological activities including anti-inflammatory, tumor necrosis factor (TNF)- α inhibitory and anti-angiogenic effects. Thalidomide and its derivatives are now widely used as potent immunomodulatory drugs (IMiDs) in the treatment of several diseases including multiple myeloma (MM) and leprosy (Hansen's disease). Furthermore, thalidomide has recently been employed in the treatment of vascular diseases. However, the precise mechanism by which thalidomide exerts its teratogenic and pharmacological effects remains unknown.

A breakthrough in thalidomide research was recently achieved with identification of its primary target cereblon (CRBN), which binds directly to thalidomide and was isolated as a thalidomide-binding protein from various cell types [1]. CRBN, originally identified as a cerebral protein associated with mild mental retardation, is a highly conserved protein that forms a CRL4-type E3 ubiquitin ligase complex with Cul4A and damaged DNA binding protein 1 (DDB1), and plays a key role in limb outgrowth and expression of fibroblast growth factor Fgf8 in zebrafish and chicks. Thalidomide initiates its teratogenic effects by binding to CRBN and inhibiting the associated ubiquitin ligase activity. Moreover, a human MM cell line with deletion of the CRBN gene is resistant to thalidomide derivatives, indicating that CRBN is involved in both the teratogenic and beneficial effects of thalidomide. We set out our collaboration of the structural works of human CRBN complexed with thalidomide (Thal) or its derivatives such as lenalidomide (Len) and pomalidomide (Pom) with the Prof. Handa group at Tokyo Medical University and the Celgene Corporation group at Saint Diego. Recently, we have reported a series of crystallographic and biochemical studies on the interaction between thalidomide and human DDB1-CRBN or mouse CRBN thalidomide-binding domain (TBD), and provide a structural basis of the binding specificity and stereospecific effects of thalidomide [2].

X-ray intensity data from our crystals were collected

at the SPring-8 synchrotron facility for the provision of synchrotron data-collection facilities (beamlines **BL41XU** and **BL44XU**), the Advanced Photon Source supported by the US Department of Energy, the Advanced Light Source operated by the Lawrence Berkeley National Laboratory and the Canadian Light Source (beamline 08ID-1) supported by the Natural Sciences and Engineering Research Council of Canada. Using these synchrotron facilities, we determined structures of the human DDB1-CRBN-Len (3.01 Å resolution), the mouse CRBN TBD-Thal (1.88 Å) and the mouse CRBN TBD-Pom (2.0 Å) complexes and the free form of mouse CRBN TBD (2.0 Å). Our structures of the TBD-drug complexes are precise enough for discussion of the specific interactions between the drug and the target protein.

The overall structure of the ternary complex (Fig. 1) reveals that CRBN binds between the DDB1 β -propellers A and C in a similar location to other DDB1-cullin 4-associated factors (DCAFs), which play a role of substrate receptors such as DDB2, DCAF9, Hbx and SV5V19. However, the nature of the interaction with DDB1 exhibits differences compared to those found in previously determined DDB1-DCAF complexes. CRBN made intimate interactions with both β -propeller A and C domains of DDB1 via a series

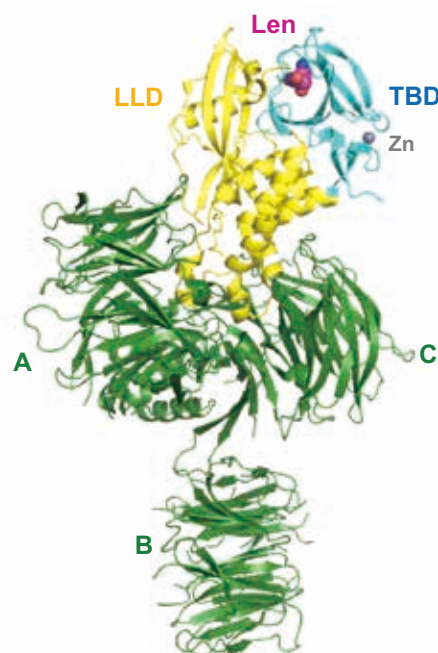


Fig. 1. Overall structure of the human DDB1–CRBN–Len complex. DDB1 (green) consists of three β -propeller domains A, B and C. CRBN has a Lon-like domain (LLD in yellow) directly bound to DDB1 and TBD (cyan) bound to Len.

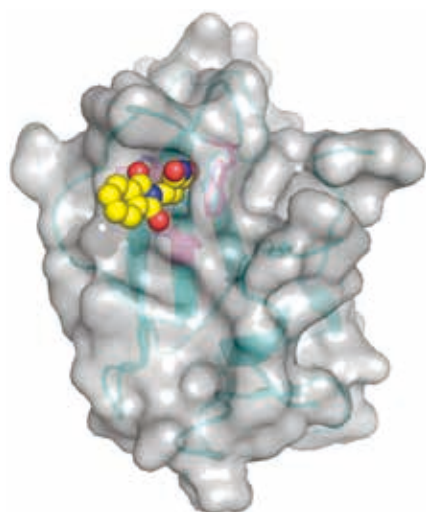


Fig. 2. Structure of TBD bound to Thal. The bound Thal molecule (a space-filling model) is docked into the binding pocket on the surface of TBD (transparency surface representation with a ribbon model).

of helices, whereas DCAFs generally position a helix-turn-helix motif in the DDB1 binding site predominantly formed by the β -propeller C domain. The structure of CRBN reveals that CRBN is a DCAF that does not exhibit WD-repeat architecture typical of the class and composed of two domains; an N-terminal Lon-like domain (LLD in yellow) directly bound to DDB1 and a C-terminal TBD (cyan) bound to Lon. The DDB1-binding motif exists in LLD and the thalidomide binding site exists in TBD. These sites are located at the opposite sides of the CRBN surface.

Interestingly, LLD and TBD are structurally distinct and in fact TBD isolated from LLD retains the binding affinity to Thal and its derivatives. High-resolution structures of TBD bound to the drugs reveal details of the specific interactions between the protein and the drug (Fig. 2). Thalidomide is a small synthetic compound, α -phthalimido-glutarimide (IUPAC systematic name, 3-(*RS*)-2-(2,6-dioxo-3-piperidyl) isoindole-1,3-dione). The phthalimido portion is largely nonpolar but glutarimide portion possesses imido group, which is capable of hydrogen bond formation. In our structures, Thal and its derivatives bind CRBN with the glutarimide portion inside of the binding pocket, which is formed by three imidazole rings from tryptophans (Fig. 3). Inside the pocket, the imido group of the glutarimide. This binding mode was also observed in the recently-reported structures of hybrid complexes of human DDB1-chick CRBN and Thal and its derivatives, while the structures were determined at a marginal resolution between 3.0 Å and 3.5 Å [3]. This binding mode is unusual since the nonpolar portion, phthalimido, of the drug is exposed to the solvent but the rather polar portion, glutarimide, is buried inside

the pocket. This paradox could be solved by the recently-clarified fact that these drugs directly bound to CRBN promote the recruitment of substrates Ikaros (IKZF1) and Aiolos (IKZF3) to the E3 complex, thus leading to substrate ubiquitination and degradation [4,5]. Thus, Thal and its derivatives play a role of an interfacial drug, such as brefeldin A acting against Arf GEF. This specific substrate recruitment is thought to be responsible to potent immunomodulatory effects of Thal and its derivatives in the treatment of several diseases including MM. In contrast to the recruitment, Thal binding to CRBN should block binding of authentic or endogenous substrates to CRBN. This inhibition may cause teratogenic effects. Further experiments should be carried out to clarify these dual activity together with structure determination of ternary and quaternary complexes of the E3 substrate and Thal with CRBN or CRBN-DDB1.

Our structural study provides a structural framework for further investigations on the mechanisms of the pharmaceutical and teratogenic actions of this drug and for the development of more effective thalidomide derivatives.



Fig. 3. A close-up view of Thal bound to the binding pocket on the TBD surface. The bound Thal molecule (a stick model in yellow) is sandwiched three side-chain imidazole rings from CRBN tryptophans (magenta).

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References

- [1] T. Ito *et al.*: Science **327** (2010) 1345.
- [2] P.P. Chamberlain, A. Lopez-Girona, K. Miller, G. Carmel, B. Pagarigan, B. Chie-Leon, E. Rychak, L.G. Corral, Y.J. Ren, M. Wang, M. Riley, S.L. Delker, T. Ito, H. Ando, T. Mori, Y. Hirano, H. Handa, T. Hakoshima, T.O. Daniel and B.E. Cathers: Nat. Struct. Mol. Biol. **21** (2014) 803.
- [3] E.S. Fischer *et al.*: Nature **512** (2014) 49.
- [4] G. Lu *et al.*: Science **343** (2014) 305.
- [5] J. Kreonke *et al.*: Science **343** (2014) 301.

Crystal structure of Cas9 in complex with guide RNA and target DNA

Microbes have the CRISPR-Cas adaptive immune system as a defense against invading foreign nucleic acids, such as phages and plasmids. The CRISPR-Cas system is composed of CRISPR (clustered, regularly interspaced short palindromic repeats) loci in the genome, and a cluster of Cas (CRISPR-associated) genes. The CRISPR loci consist of identical repeating sequences (referred to as repeats) interspaced by short sequences (referred to as spacers) derived from previously infected foreign nucleic acids. There are three types of CRISPR-Cas systems (types I–III). In the type II CRISPR-Cas system, dual noncoding RNAs, crRNA (CRISPR RNA), and tracrRNA (*trans*-activating crRNA), are transcribed from CRISPR loci, and bind to the effector nuclease Cas9 to form a Cas9–crRNA–tracrRNA ternary complex [1]. The ternary complex recognizes the target double-stranded DNAs through sequence complementarity between the target DNA and the 20-nt guide sequence in the bound crRNA, inducing a DNA double-strand break in the target DNA (Fig. 1). In addition to base pairing between the sgRNA and the target DNA, a short nucleotide motif located adjacent to the cleavage site of the target DNA (referred to as PAM (protospacer adjacent motif)) is necessary for the Cas9-catalyzed DNA cleavage [2]. PAMs differ among the Cas9 proteins. For instance, Cas9 from the pathogenic bacterium *Streptococcus pyogenes*, which is widely utilized for genome-editing technology,

recognizes 5'-NGG-3' as the PAM. The 20-nt guide sequences in crRNAs are derived from previously infected foreign nucleic acids, so that crRNAs serve as molecular memories.

In 2012, biochemical studies revealed that Cas9 is an RNA-guided DNA endonuclease with two nuclease domains (HNH and RuvC) [2] (Fig. 2(a)). The HNH domain cleaves the DNA strand complementary to the 20-nt guide sequence in the crRNAs (cDNA), while the RuvC domain cleaves the noncomplementary DNA strand (ncDNA) (Fig. 1). A synthetic single guide RNA (referred to as sgRNA), in which crRNA and tracrRNA are fused with a tetraloop, can also direct Cas9 to DNA cleavage (Fig. 1). Since the discovery in 2013 that the Cas9–sgRNA system can induce site-specific DNA double-strand breaks in the genome [3], the Cas9–sgRNA system has been attracting much attention as a new, versatile genome-editing technology, which works effectively in various types of cells and organisms. Catalytically dead or inactive Cas9 (referred to as dCas9) can serve as an RNA-guided genome-targeting platform, and dCas9-based new technologies, such as those for transcription regulation and chromatin imaging, have also been developed. Despite the rapid advancements in Cas9-based technologies, the mechanism by which the Cas9–sgRNA complex recognizes and cleaves the target DNA remains elusive since Cas9 shares no sequence similarity with other known proteins, except for the two nuclease domains.

To understand this unprecedented, RNA-guided DNA cleavage mechanism, we sought to solve the crystal structure of the Cas9–sgRNA–cDNA ternary complex. *S. pyogenes* Cas9 was expressed in *Escherichia coli*, and then purified to homogeneity by column chromatography. sgRNA was transcribed *in vitro* using T7 RNA polymerase, and then purified by denaturing polyacrylamide gel electrophoresis. The reconstituted Cas9–sgRNA–cDNA ternary complex was purified on a size-exclusion column and subsequently crystallized by the vapor diffusion method. X-ray diffraction data were collected at beamlines BL32XU and BL41XU, and the crystal structure of the ternary complex was determined at 2.5-Å resolution by the Se-SAD method [4]. The crystal structure revealed that Cas9 adopts a bilobed architecture (Fig. 2(b)). One lobe comprises a number of α helices and is responsible for the recognition of sgRNA and cDNA. Thus, we named it the recognition (REC) lobe. Because the other lobe contains the

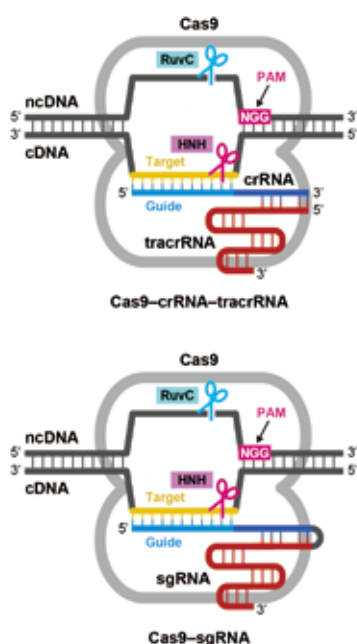


Fig. 1. RNA-guided DNA cleavage by Cas9.

HNH and RuvC nuclease domains, we named it the nuclease (NUC) lobe. The REC and NUC lobes are connected by a long α helix (named the bridge helix) and a flexible linker. In the structure, the HNH domain is located away from the cDNA cleavage site, indicating that the present structure represents an inactive form, and that the HNH domain undergoes a conformational change to approach and cleave the cDNA during catalysis. The negatively charged sgRNA:cDNA heteroduplex is accommodated in a positively charged groove between the REC and NUC lobes, and is recognized by Cas9 via the sugar-phosphate backbone of the heteroduplex. This explains the sequence-independent recognition by Cas9. The NUC lobe also contains a carboxy-terminal domain, which is located at a position suitable for interacting with the PAM. We named this the PAM-interacting (PI) domain. Indeed, our mutational analysis revealed that the PI domain participates in PAM recognition. As anticipated from their amino acid sequences, the REC lobe and the PI domain adopt new protein folds.

The ternary complex structure also reveals that the sgRNA forms a T-shaped architecture, which is composed of the sgRNA:cDNA heteroduplex, the repeat:anti-repeat duplex, and three stem loops (stem loops 1–3), where stem loops 1 and 2 are connected

by a single-stranded linker (Fig. 2(b)). The repeat:anti-repeat duplex adopts a partial duplex architecture, which is extensively recognized by the REC lobe. The PAM-proximal ‘seed’ region in the 20-nt guide sequence is extensively recognized by a cluster of conserved arginine residues on the bridge helix (Fig. 2(b)), which is consistent with the previous data showing the importance of the base pairing between the ‘seed’ region in sgRNA and the cDNA for Cas9-catalyzed cleavage [2].

Based on the structural finding that the tetraloop and stem loop 2 in sgRNA do not directly contact Cas9 and are exposed to the solvent in the ternary complex [4] (Fig. 2(b)), we recently developed a potent, dCas9-based transcription activator [5]. We generated an engineered sgRNA, in which the MS2-interacting hairpin aptamers are fused to the tetraloop and stem loop 2, and showed that three components—dCas9, the engineered sgRNA, and the MS2 protein fused with transcription activator domains—are able to mediate efficient, targeted transcriptional activation of endogenous genes.

In summary, the high-resolution structure of the Cas9–sgRNA–cDNA complex provides mechanistic insight into the RNA-guided DNA cleavage by Cas9, and thus, paves the way for the rational design of new, Cas9-based technologies.

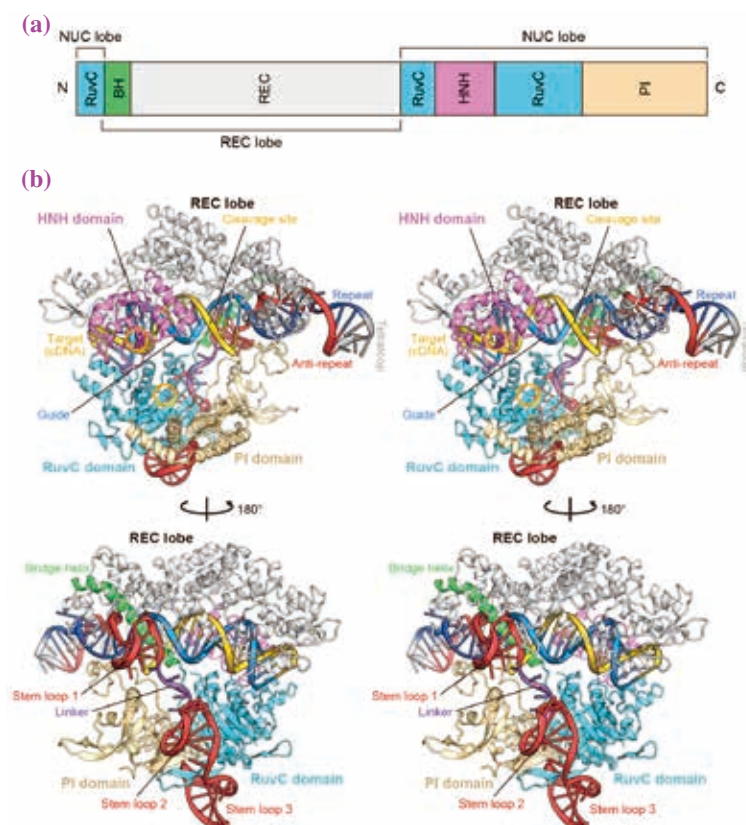


Fig. 2. (a) Domain structure of Cas9. (b) Crystal structure of the Cas9–sgRNA–cDNA ternary complex (stereoview). The active sites are indicated by orange circles. Structural image was prepared with CueMol (<http://www.cuemol.org>).

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References

- [1] E. Deltcheva *et al.*: Nature **471** (2011) 602.
- [2] M. Jinek *et al.*: Science **337** (2012) 816.
- [3] L. Cong *et al.*: Science **339** (2013) 819.
- [4] H. Nishimasu, F.A. Ran, P.D. Hsu, S. Konermann, S.I. Shehata, N. Dohmae, R. Ishitani, F. Zhang and O. Nureki: Cell **156** (2014) 935.
- [5] S. Konermann *et al.*: Nature **517** (2015) 583.

Blue light-induced conformational changes in *Chlamydomonas* phototropin

In 1880, Charles Darwin, together with his son Francis, wrote a book 'The power of movements in plants' in which they reported observations on various types of tropic responses of plants. One of the responses that received a great deal of attention is the phototropic response where plants display a curvature response to the direction of light, which is known as phototropism. One hundred ten years later, phototropin1 (phot1) was discovered in *Arabidopsis thaliana* (At) as a blue-light (BL) receptor protein that causes the phototropism in plants. Since then, phototropins (phot) have been identified in many organisms from *Chlamydomonas* to higher plants based on the sequence homology to phot1. In particular, the phototropin (phot) found in *Chlamydomonas reinhardtii* (Cr) is used as a model to study the molecular architecture and mechanism for the initial step of phototropism. In higher plants, phototropins are known to act as receptors for the phototropic response as well as mediate chloroplast relocation, stomata opening, leaf flattening, and leaf photomorphogenesis. All of these responses serve to optimize the efficiency of photosynthetic activities.

Phot is a blue-light-regulated protein kinase, and comprises approximately 700-1000 amino acid residues and two flavin mononucleotide (FMN) molecules. Phot folds into three functional domains, two light-oxygen-voltage sensing domains (designated as LOV1 and LOV2) in the N-terminal half and one Ser/Thr kinase domain (STK) in the C-terminal half (Fig. 1). Each LOV non-covalently binds one FMN to receive blue-light. Upon BL excitation, LOV undergoes a cyclic photoreaction with a small conformational change in the protein moiety. Currently, the mechanism to activate the STK is being investigated in order to understand the mechanism to convert physical light stimuli into biochemical cellular signals through signaling cascades initiated by the photo-induced phosphorylation of STK.

We have been studying the molecular structure of Cr phot to understand the molecular mechanism underlying the BL-dependent activation of the kinase [1-3]. Currently, we are studying the domain organization and BL-dependent conformational changes of Cr phot [4] using small-angle X-ray scattering (SAXS) conducted at beamline BL45XU and a PILATUS3 detector (DECTRIS, Switzerland). The X-ray wavelength was 0.9000 Å, and the camera distance was approximately 2000 mm. Each SAXS pattern was collected as a set of time-resolved 18 frames during an exposure of 60 s. For each

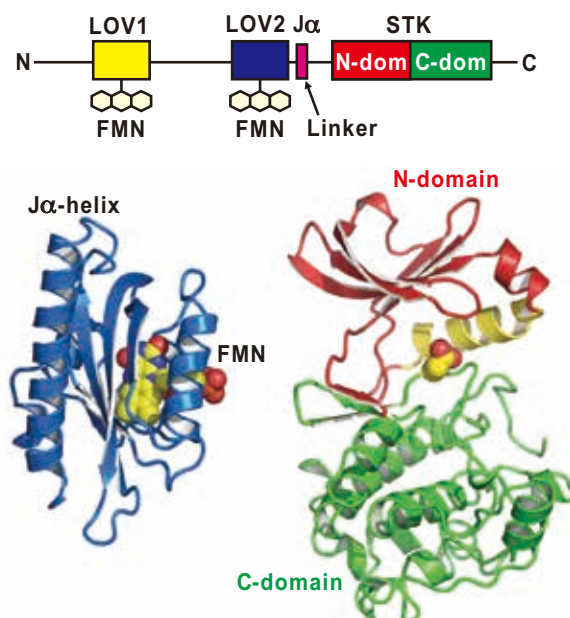


Fig. 1. Schematics of the domain structures of phot, and the homology models of LOV2 and STK.

sample, SAXS profiles were measured sequentially in the dark, under BL with the fluence rate of 450 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ after pre-irradiation for 5 min, and again in the dark after adapting to the dark for more than 5 min. The SAXS profiles after the subtraction of the background scattering and normalization regarding the concentration were subjected to analyses using Guinier's plot, the distance distribution function, and the *ab initio* construction of low-resolution molecular models.

SAXS profiles display BL-dependent changes. Figure 2 shows a typically example. Owing to the almost monodisperse characteristics under both dark and light conditions, the apparent molecular weight is estimated to be 70,000, which is as large as that determined by size exclusion chromatography. Thus, Cr phot exists in the monomeric form in solution. The radius of gyration (R_g) values in infinitely diluted conditions are 42.7 Å in the dark and 43.4 Å under BL irradiation. The maximum dimension estimated from the $P(r)$ function in the dark is 157 Å, which is smaller than the 167 Å under BL. These changes with regard to the molecular dimensions indicate a BL-induced extension of molecular size.

The molecular model in the dark appears as an elongated shape with dimensions of approximately $140 \times 40 \times 40 \text{ \AA}^3$. The main body is very similar to that of the LOV2-kinase fragment from *Arabidopsis phot2* with respect to its size and shape. Moreover, the crystal structure of LOV1 fits well with the tip of the molecular model. The molecular model under BL is similar to that in the dark but the LOV1 part under BL a different relative position. The differences in the molecular models suggest that the positions and orientations of the LOV2-LOV1 region would be light dependent (Fig. 3 right). The present SAXS studies are the first report on the whole structure of Cr phot and the position of the LOV1 relative to LOV2-STK.

From spectroscopic studies, the $J\alpha$ helix connecting LOV2 and STK unfolds and dissociates from LOV2. This structural change likely plays a key role in the activation of STK. In the previous SAXS study on the LOV2-kinase fragment of *At phot2*, we observed BL induced a separation of the LOV2 from STK. Based on that observation, we proposed the photoactivation mechanism of STK where the light-activated LOV2 domain triggers conformational changes in the linker region, including $J\alpha$ between LOV2 and KD, and the structure of which is in an intrinsically unfolded

state. In addition, our biochemical study indicates that the amino acids around the N-terminus of LOV2 also play essential roles in the photoactivation of the kinase. In the crystal structure of LOV2- $J\alpha$ of *At phot1*, $J\alpha$ and the small helix are in contact. Therefore, the linker region and the N-terminal small helix would act as enhancers to transmit the small conformational changes in LOV2, modifying the conformation of STK in the BL-induced intramolecular signaling. Further details of the structure of Cr phot in the dark must be studied by crystal structure analysis in the near future.

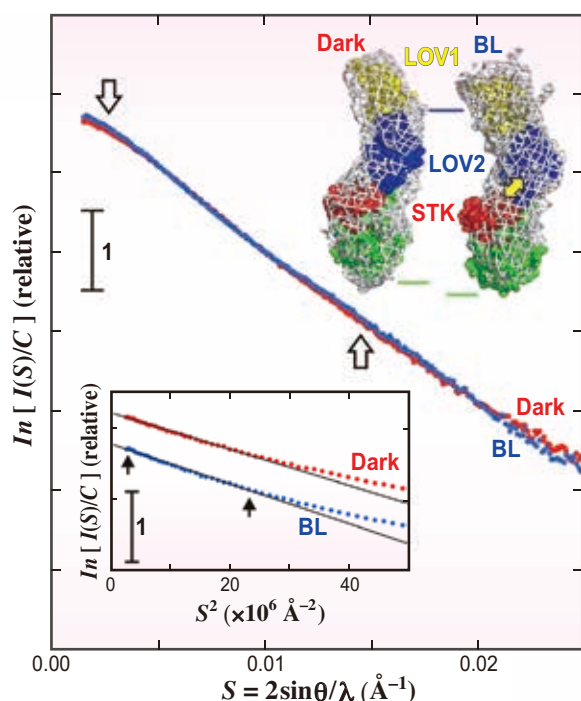


Fig. 2. SAXS profiles and their Guinier plots (inset) in the dark (red dots) and under BL irradiation (blue dots). Restored molecular models of CrPful in the dark (red colored mesh) and under BL (blue colored mesh) are shown in the right panel. Low-resolution molecular models (mesh) of CrPful in the dark and under BL irradiation. Homology modeled Ser/Thr kinase (N-lobe: red, C-lobe: green), LOV2 and the crystal structure of LOV1 (yellow) are fitted to the restored SAXS models.

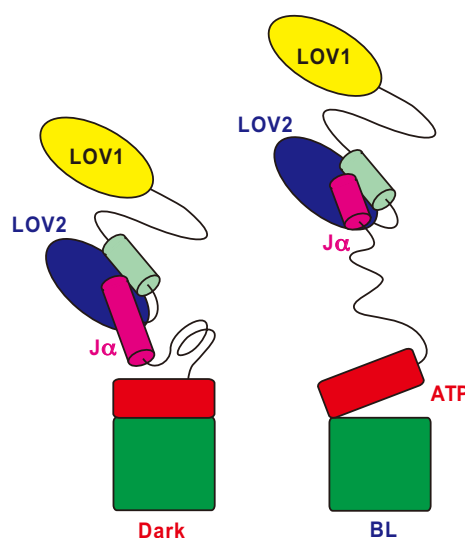


Fig. 3. Schematic illustration of the molecular organization of LOV1, LOV2 and STK as well as the $J\alpha$ (pink) and N-terminal small helix (green) in a monomeric Cr phot molecule and its BL-induced changes.

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References

- [1] M. Nakasako *et al.*: *Biochemistry* **43** (2004) 14881.
- [2] M. Nakasako *et al.*: *J. Mol. Biol.* **381** (2008) 718.
- [3] Y. Takayama *et al.*: *Biochemistry* **50** (2011) 1174.
- [4] K. Okajima, Y. Aihara, Y. Takayama, M. Nakajima, S. Kashojiya, T. Hikima, T. Oroguchi, A. Kobayashi, Y. Sekiguchi, M. Yamamoto, T. Suzuki, A. Nagatani, M. Nakasako and S. Tokutomi: *J. Biol. Chem.* **289** (2014) 413.

Real time intramolecular motion of ligand-gated ion channel at the single molecule level

Most soluble proteins are composed of several subunits and the protein's function can be expressed by changing its intra-structure or its relative position in a multimeric protein. Moreover, 80% of drug targets are membrane proteins, which are composed of several subunits. Therefore, it is important to obtain information about such cooperative motions of each subunit in order to understand the mechanism of functional proteins. Because cooperative motions are thought to be small (on the atomic scale) and fast (on the microseconds scale), a technique to acquire such dynamic information is desired in both academic and industry.

We have proposed a single molecule technique that utilizes short wavelength probes (X-rays) to monitor the internal motions of a single protein. We call it diffracted X-ray tracking (DXT), and it would be a powerful technique in biological sciences to detect atomic-scale dynamic motions of a protein on the single molecule level with several tens of microseconds time resolution. Figure 1 illustrates DXT. A protein is labeled with a nanocrystal, with a size of several tens of nanometers, and the motions of the nanocrystal coupled with the protein's motions are recorded as trajectories of the Laue spots from the nanocrystal. Professor Yuji C. Sasaki designed this technique in 1997 and presented it in 2000 at SPing-8 [1].

Here, using DXT with an improved time resolution at the high-flux beamline BL40XU [2], we performed real-time 100- μ s-time resolution single molecule observations of nAChR from Torpedo, which is one of the most thoroughly studied members of the pentameric ligand-gated ion channel (pLGIC) family, and AChBP, a homologue to the extracellular ligand-binding domain of nAChR that is often used as a model for the ligand-binding domain of pLGICs. Based on an electron crystallographic structural study, it was hypothesized that the conformational changes associated with channel opening of nAChR would cause twisting and tilting motions in both the ligand-binding and channel domains. The DXT method can provide such information about the target proteins, as shown in Fig. 1(c). Thus, we applied it to investigate the gating mechanism of nAChR.

We immobilized a gold nanocrystal on the C-terminus of AChBP via a Met-tag (Fig. 1(a)) and on the extracellular side of nAChR via an antibody (Fig. 1(b)). Then we recorded the rotational motion of the gold nanocrystal on the target protein around two axes, tilting (θ) and twisting (χ), and compared the distributions under different experimental conditions, e.g., in the presence of an activator (acetylcholine: Ach), in the presence of a deactivator (α -bungarotoxin: α Btx),

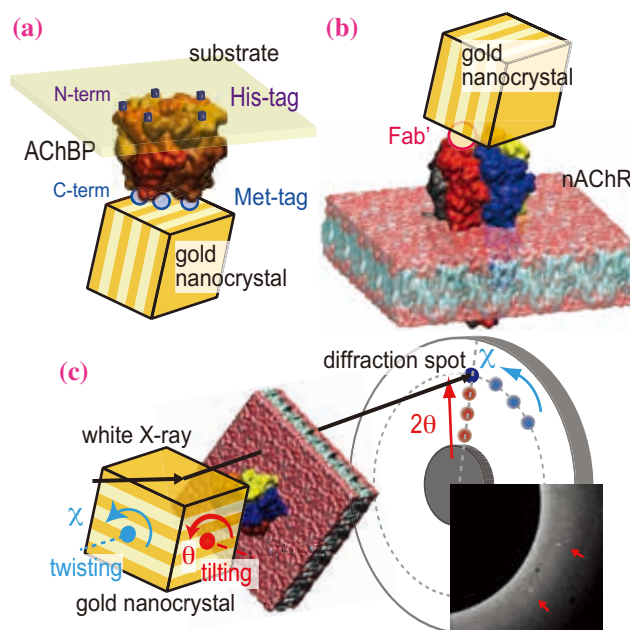


Fig. 1. DXT measurements of AChBP and nAChR. Schematic of the immobilization of gold nanocrystals on (a) AChBP and (b) nAChR. (c) Schematic of the tilting (θ) and twisting (χ) motions observed by DXT. [2]

and in the absence of any ligands. **Figures 2(a) and 2(b)** show the two-axis differential (ACh- α Btx) internal motion histograms of AChBP and nAChR, respectively, over a time interval of 100 μ s. In **Fig. 2(a)**, the ACh-positive area in the yellow-red color scale is distributed in the upper right, and the α Btx-positive area in the yellow-blue color scale is distributed in the lower left of the map. These results indicate that both the tilting and twisting motions of AChBP are facilitated in the presence of ACh, but inhibited in the presence of α Btx. AChBP is a homopentamer, and the ACh binding site is located at the boundary between subunits. The nanocrystal is labeled in the region sensitive to conformational changes upon ACh binding. Therefore, the enhanced nanocrystal motion, which is observed in the presence of ACh by DXT, reflects the conformational change of the ACh binding site of AChBP upon binding and unbinding of ACh. **Figure 2(b)** shows the differential (ACh- α Btx) internal motion histograms of nAChR, which are more

complicated. The histogram can be divided into three areas as illustrated in right column of **Fig. 2(b)**. Torpedo nAChR is a heteropentamer with two ACh binding sites. Additionally, prolonged exposure to ACh induces nAChR to adopt a desensitized state, indicating that nAChR can exist in multiple conformations. We hypothesize that the complex motion pattern observed by DXT is attributed to these heterogeneous characteristics of nAChR. Although we could not discern the motion pattern of the nAChR states in the current data set, ACh-induced conformational changes in the α -subunit of nAChR are clearly observed by our method.

The DXT technique can be used to monitor the dynamic behavior of a single molecule at all principal sites for intramolecular dynamics with an immobilizing motion tracer at the site. This technique is also expected to greatly contribute to the discovery of allosteric drugs without side effects because intramolecular dynamic information is essential to realize such drugs.

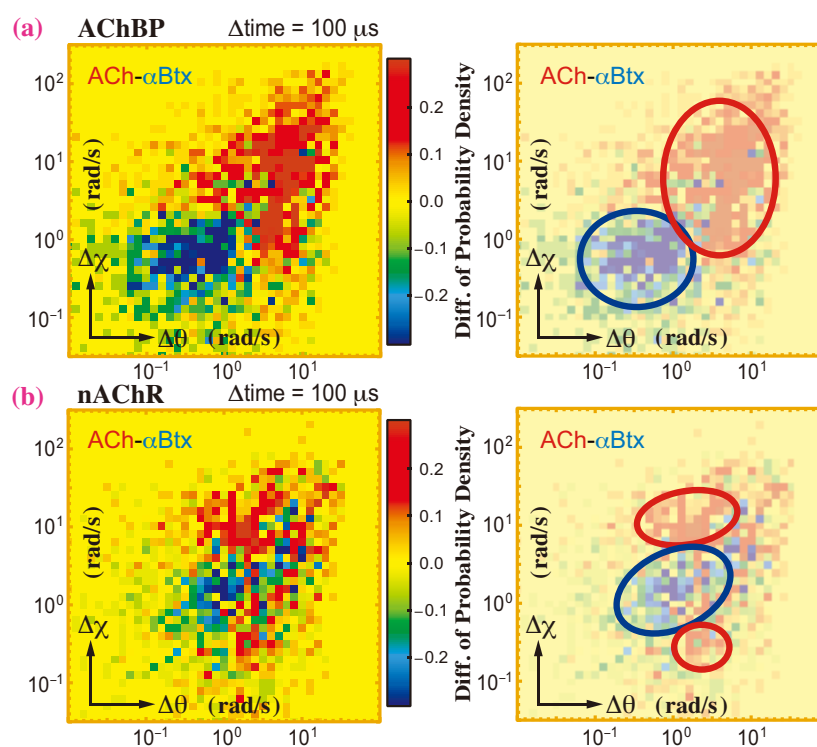


Fig. 2. Difference internal motion 2D histograms of AChBP and nAChR. [2]

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References

- [1] Y. Sasaki *et al.*: Phys. Rev. E **62** (2000) 3843.
- [2] H. Sekiguchi, Y. Suzuki, Y. Nishino, S. Kobayashi, Y. Shimoyama, W. Cai, K. Nagata, M. Okada, K. Ichiiyanagi, N. Ohta, N. Yagi, A. Miyazawa, T. Kubo and Y.C. Sasaki: Sci. Rep. **4** (2014) 6384.

Role of Rho-kinase in early diabetic coronary vasoconstriction

The coronary circulation is vulnerable to diabetic complications independent of atherosclerosis. Chronic hyperglycemia causes angiopathy. This leads eventually to congestive heart failure. Abnormal coronary blood flow regulation develops in disease states when the factors that maintain muscular arteries open, vasodilators or relaxing factors, are not produced in sufficient quantities by the lining of cells in the lumen of the vessels called the endothelium to counterbalance an increase in constrictor factors. We have shown that basal endothelium dependent vasodilation is maintained in early diabetes (type-1), but focal stenoses and segmental constrictions occur when prostacyclin and nitric oxide (NO) production are prevented [1]. Thus the diabetic coronary circulation is vulnerable to ischemia when dilator bioavailability is reduced. The RhoA/Rho-kinase (ROCK) pathway is implicated in hypertension, angina and vasospasm [2].

Our objective was to determine if an increase in ROCK mediated constriction plays a role in the onset of coronary vascular dysfunction in type-1 diabetes. In this study we utilized microangiography with synchrotron radiation from the beamline **BL28B2** as the X-ray source to investigate the constrictor tone present in the *in vivo* coronary circulation of Sprague-Dawley rats from the microvessels to large arteries [3]. Briefly, male rats were made diabetic with streptozotocin (65 mg/kg) and three weeks later we surgically prepared them under anaesthesia for closed chest imaging at physiological heart rates. Cine-angiograms were recorded during drug administrations for ~3 seconds as an iodine contrast agent was infused through a cannula inserted into the aorta via the right carotid artery. Up to four branching orders of arterial vessels were visualized in diabetic and control rats (internal diameter 40-300 μm), including the microvessels that are the main source of vascular resistance (Fig. 1). Vessel diameter and vessel number were quantified and compared during various stimulations with vasodilators (including acetylcholine, ACh), following complete inhibition of prostacyclin and NO production (combined blockade) and subsequent administration of the ROCK inhibitor fasudil (10 mg/kg).

Diabetic rats had significantly elevated blood glucose and lower heart rates, but did not differ in baseline coronary vessel calibre across the first 3 arterial branching segments (Fig. 1). Pronounced dilation in response to ACh infusion was found in both groups in the presence of NO and prostacyclin,

but widespread segmental constrictions (white arrows, Fig. 1(g)) in their absence in diabetic rats. ROCK inhibition during nitric oxide synthase and cyclooxygenase blockade restored or dilated vessel calibre relative to baseline in diabetic rats (Fig. 1(h)). A novel finding in this study is the *in vivo* identification of ROCK as a likely mediator of localised constrictions in diabetic hearts. This is supported by the fact that acute ROCK inhibition greatly reduced the incidence of segmental constrictions in second and third

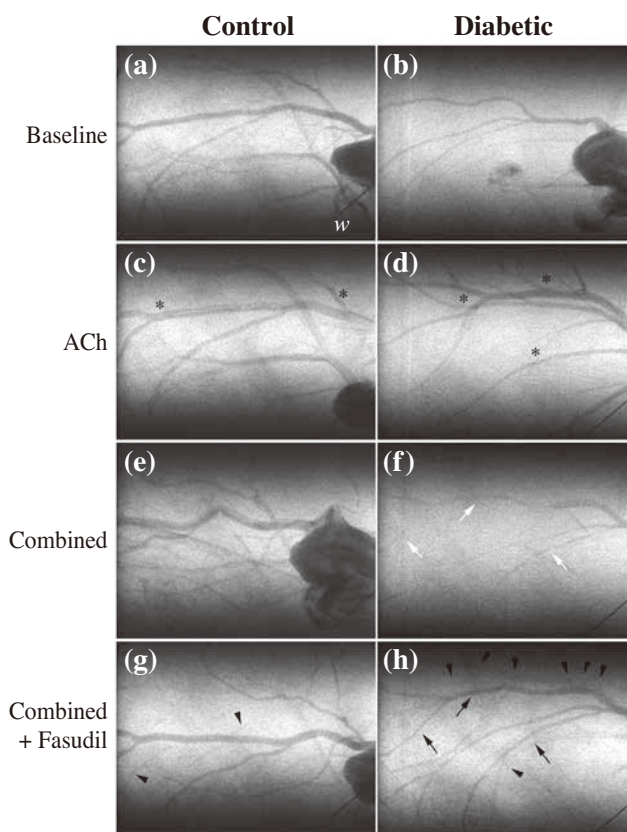


Fig. 1. Representative coronary angiograms in control and diabetic rats [3]. Baseline vehicle lactate infusion (a & e), ACh infusion (b & f: 3.0 $\mu\text{g/kg/min}$), following administration of L-NAME and meclofenamate (combined: c & g) and subsequent fasudil treatment (d & h: 10 mg/kg intravenous). (a & e) Baseline response to lactate infusion. (b & f) Control and diabetic, large vessels maintained vessel diameter greater than baseline (black asterisks). (c) Control, vessel diameter maintained. (g) Diabetic segmental vasoconstriction (white arrows). (d) Fasudil response in control, vessel diameter maintained. (h) Vasodilation of medium and small arteries (black arrows). w: 50 μm tungsten wire.

order vessels following combined blockade (Fig. 2). Furthermore, there was a trend towards increased ROCK2 activation (Fig. 3). Based on nitrotyrosine staining, oxidative stress was not significantly elevated in early diabetic rats. Since there was neither an increase in perivascular fibrosis nor an increase in intimal thickening of coronary arteries (confirmed by immunohistochemistry) we attribute the increase in vasoconstriction after blockade to functional upregulation of Rho-kinase activity, rather than structural remodeling of the heart.

These findings demonstrate that an increase in vasoconstrictor tone mediated by Rho-kinase influences coronary perfusion in the early stages of diabetes, and is therefore likely to contribute to vulnerability to ischemia when vasodilator bioavailability becomes diminished in later stages. Notably, ROCK-mediated vasoconstriction was not a consequence of increased oxidative stress, inferring that it was most likely mediated by increased activation of myosin light chain phosphatase in the arterial smooth muscle cells. Moreover, this microangiography approach demonstrates that fasudil treatment might improve the diabetic coronary circulation in the long term. Since this study was published a clinical small trial has now shown that indeed, chronic treatment with fasudil also improves cardiac dysfunction in patients with advanced diabetes [4].

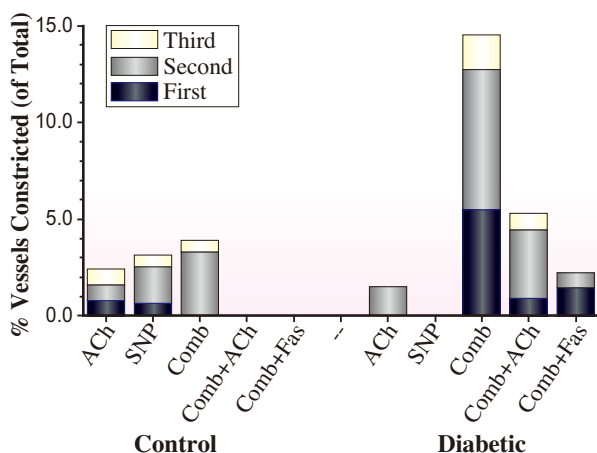


Fig. 2. Occurrence of segmental constrictions in control and diabetic rats in relation to treatment and arterial branching order. Segmental constrictions (most of segment length constricted relative to baseline) are expressed as a percentage of total visible vessels. Control, $n=7$ and diabetic, $n=8$.

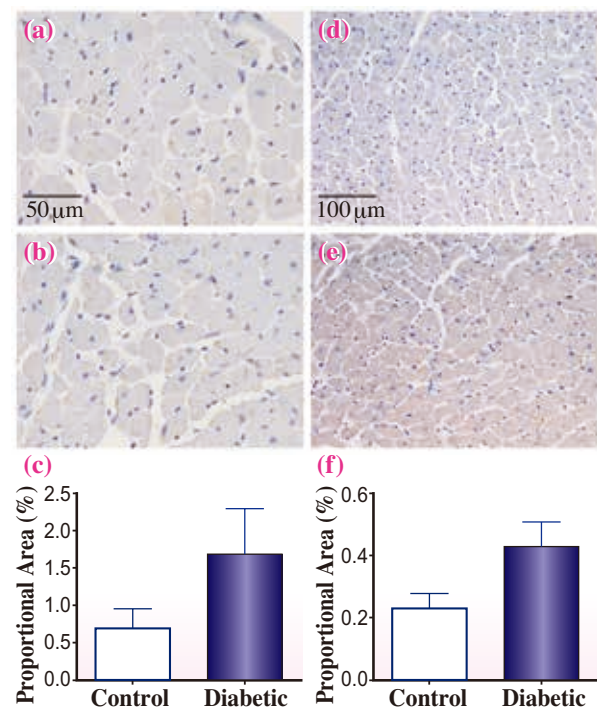


Fig. 3. Myocardial ROCK1 and ROCK2 immunohistochemistry in control and diabetic rats. ROCK1 expression (e) in control (a) and diabetic (b) rats (40x objective). ROCK2 expression (f) in control (c) and diabetic (d) rats (20x objective). ROCK1 expression was not significantly different between control and diabetic groups, although there was a trend towards increased ROCK1 expression in diabetic rodents. ROCK2 expression was borderline significantly increased ($P<0.053$) in diabetic compared to control animals. Control, $n=6$ and diabetic, $n=7$. Values expressed as mean $\pm$ SEM.

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References

- [1] M.J. Jenkins *et al.*: Arterioscler. Thromb. Vasc. Biol. **32** (2012) 370.
- [2] H. Noma *et al.*: Am. J. Physiol. Cell Physiol. **290** (2006) C661.
- [3] J.T. Pearson, M.J. Jenkins, A.J. Edgley, T. Sonobe, M. Joshi, M.T. Waddingham, Y. Fujii, D.O. Schwenke, H. Tsuchimochi, M. Yoshimoto, K. Umetani, D.J. Kelly, M. Shirai: Cardiovasc. Diabetol. **12** (2013) 111.
- [4] R. Guo *et al.*: Heart Vessels (2014) 1.

Ferrocene-based metal-organic nanotubes and nanorings with ultra-large dimensions: Redox-mediated modulation of noncovalent forces

Cleavage and formation of covalent bonds at desired positions are modern key technologies for the development of complicated organic molecules. However, when it comes to the synthesis of nanoarchitectures utilizing relatively weak attractive forces between molecules (i.e., noncovalent interactions), such nanoscale manipulations in which the self-assembled structures are deconstructed into modules and reassembled can be challenging due to difficulties in selectively modulating certain interactions among various attractive forces. Although a variety of beautiful nanostructures have been noncovalently synthesized, even more exotic or inaccessible self-assembled motifs may become available if methods to cut, paste, or merge assembled structures are realized. Some nanostructures have been shown to break into thermodynamically inaccessible low-symmetry pieces [1-3]. However, such selective cutting requires prior stabilization of the parent nanostructures by partial covalent crosslinking.

We have developed out-of-box supramolecular nanoarchitectures that can be cut and pasted without the aid of covalent crosslinking [4]. The nanoarchitecture is an ultralarge-diameter metal-organic nanotube, which can be preferentially 'cut' into its constituent coordination nanorings. Each nanoring has a 200-membered decagonal structure with an extra-large diameter of 7.5 nm. The key to successfully 'cut' the nanotube is the redox-active ferrocene motif employed in our metal-ligating units **FcL₁** and **FcL₂** (Fig. 1), which possess a metal-coordinating 4-pyridyl group at each terminus of the four aromatic arms. Since ferrocene derivatives thermally rotate their cyclopentadienyl rings freely in solution, both ligands change their tetratopic geometry dynamically. This 'geometrical uncertainty' should give rise to the formation of various types of metal-organic architectures, including fibers, macrocycles, sheets, porous networks, and ill-defined aggregates. To our surprise, an assembly of **FcL₁** with AgBF₄ exclusively yields ultra-large-diameter nanotube **FcNT₁**. To elucidate the structure of these unprecedented metal-organic nanotubes, we performed small-angle X-ray scattering (SAXS) and wide-angle X-ray diffraction (WAXD) analyses of the as-prepared dispersions and dried films of the nanotubes, respectively, using synchrotron radiation at BL45XU in collaboration with Dr. Takaaki Hikima and Prof. Dr. Masaki Takata (RIKEN).

Transmission electron microscopy (TEM) of an air-dried acetonitrile (MeCN)/water dispersion of **FcNT₁** clearly displays hollow cylinders with a uniform 'wall

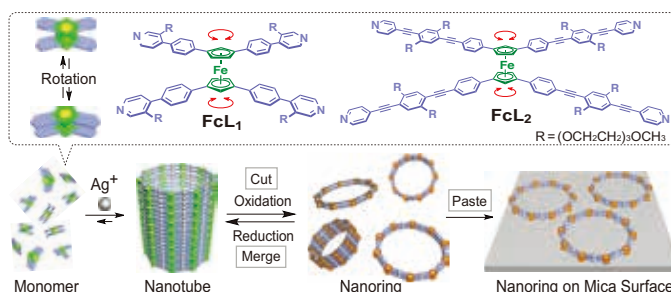


Fig. 1. Molecular structures of ferrocene-based tetratopic pyridyl ligands and schematic representation of the formation and transformation processes of ferrocene-based metal-organic architectures.

center-to-wall center' distance (nanotube diameter) of 7.5 nm [4]. SAXS analysis of **FcNT₁** in MeCN provides detailed information about the assembly dimensions. The SAXS profile has a distinct oscillation lasting to a large scattering vector q , indicating that **FcNT₁** has a uniform diameter (Fig. 2(a)). The observed SAXS pattern is nicely fitted by the form factor of a hollow cylinder model with a diameter of 7.5 nm. Considering the bending angle of **FcL₁** (144°), this nanotube diameter allows a molecular model with a decagonal (10-mer) cross-section (vertex angle; 144°) composed of 10 bent-shaped **FcL₁** ligands and 20 Ag(I) ions to be constructed (Fig. 2(c)). We also constructed nanotubular molecular models with nonagonal (9-mer) and undecagonal (11-mer) cross-sections, and found that their calculated diameters of 6.8 and 8.2 nm, respectively, do not match the observed one. Furthermore, the SAXS patterns simulated for the corresponding hollow cylinder models clearly differ from that actually observed for **FcNT₁**. These results are reasonable considering the fact that the ideal N–Ag(I)–N coordination angle of 180° can be realized only in the decagonal model. Analogous to **FcL₁**, longer-armed **FcL₂** exclusively forms metal-organic nanotubes with large and uniform diameters. The theoretical fitting of the oscillatory SAXS pattern (Fig. 2(b)) using a hollow cylinder model indicates that this nanotube has a uniform diameter of 13.3 nm, which matches the predicted value from a 360-membered decagonal cross-section (Fig. 2(c)). This diameter is the largest among known metal-organic nanotubes.

Although SAXS analyses strongly suggested that the ferrocene nanotubes possess the decagonal cross-sections, the wall structures of the tubular assemblies remain unclear. Thus, WAXD measurements of the oriented films were conducted to estimate how the

assembled components are arranged in the nanotube. We attempted several different methods to obtain such an oriented film from **FcNT₁**, and finally found that **FcNT₁** nanotubes align under a 10-T (tesla) magnetic field. This film displays a distinct WAXD pattern (Fig. 3(b)), which features a set of diffuse spots along the direction of the applied magnetic field. These spots agree well with those expected for a 2D hexagonal lattice with a lattice parameter $a = 8.7$ nm, indicating that the nanotubes are oriented perpendicular to the applied magnetic field and are hexagonally packed with an interpore distance of 8.7 nm (Fig. 3(a)). Meanwhile, along the longer axis of **FcNT₁**, two distinct diffraction arcs (Fig. 3(b)) with d -spacings of 0.70 and 0.35 nm are observed. Taking into account the molecular structure and dimensions of bent-shaped **FcL₁**, this diffraction pattern is attributed to the wall structure illustrated in Fig. 3(a), where **FcL₁** with an eclipsed geometry stacks normally on top of each other in order to maximize the intermolecular π -stacking and metallophilic interactions.

The results of these analyses suggest that **FcNT₁** is likely composed of uniaxially stacked giant nanorings.

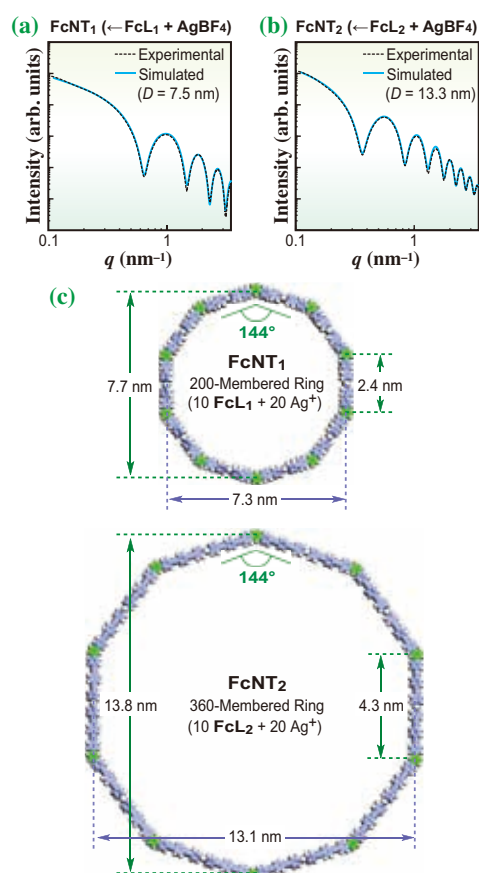


Fig. 2. (a, b) Experimental SAXS profiles (black broken curves) of MeCN dispersions of (a) **FcNT₁** and (b) **FcNT₂** at 25°C and their simulated scattering patterns (blue solid curves) for hollow cylinder models. (c) Computer-generated molecular (CPK) models for the cross sections of **FcNT₁** and **FcNT₂** using the optimized ligand structures from the molecular mechanics calculations.

The ferrocene units are located side-by-side along the longer axis of the nanotube, where stacking of the π -conjugated arrays, which include the ferrocene joint, together with the **Ag(I)–Ag(I)** metallophilic interaction, play crucial roles in the uniaxial connection of the coordination nanorings. This notion prompted us to envision that a one-electron oxidation/reduction of the ferrocene units in the nanotube may preferentially allow electrostatic modulation of the nanoring–nanoring interaction without breaking the **Ag(I)–N** coordination bond. In fact, we successfully (1) cut **FcNT₁** into the constituent nanorings by oxidation, (2) deposited the polycationic nanorings onto a negatively charged inorganic substrate, and (3) reconstructed **FcNT₁** through uniaxial stacking of the reduced nanorings. This new approach of cutting and pasting on the nanoscale level should substantially contribute toward the science and technology of highly complex and functional nanoarchitectures.

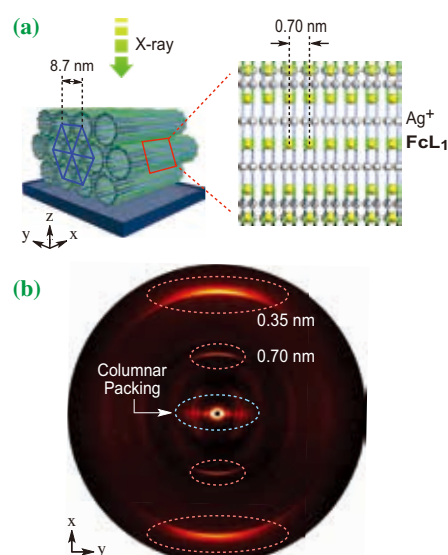


Fig. 3. (a) Schematic representation of a one-dimensionally oriented **FcNT₁** film sample and its proposed wall structure. (b) WAXD pattern of a one-dimensionally oriented **FcNT₁** film sample.

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References

- [1] R. Erhardt *et al.*: *Macromolecules* **34** (2001) 1069.
- [2] P.A. Rupar *et al.*: *Science* **337** (2012) 559.
- [3] A.H. Gröschel *et al.*: *J. Am. Chem. Soc.* **134** (2012) 13850.
- [4] T. Fukino, H. Joo, Y. Hisada, M. Obana, H. Yamagishi, T. Hikima, M. Takata, N. Fujita and T. Aida: *Science* **344** (2014) 499.

Spatially resolved structure and spectroscopic analysis of a plastically bent molecular crystal

The general perception of organic crystals being fragile and brittle has been disclaimed by the recent observation of exceptional malleability and flexibility in certain organic crystals that bend similar to polymers upon the application of external pressure [1,2]. This bending is rooted in the sliding of the slip planes in the crystal lattice, which are held together by weak intermolecular interactions such as halogen-halogen interactions or halogen-heteroatom interactions [2,3]. The rapid elastic response from the crystal under external stress can be utilized as a physical platform for energy transducing materials to develop shape-adaptive and self-actuating devices [4]. Herein with a simple organic crystal of hexachlorobenzene (HCB), we demonstrate the bending mechanism and the molecular level structural consequences of plastic deformation of a crystal under externally applied local pressure [5].

Crystals of HCB can sustain mechanical force when local pressure is applied on the (001) face and bend up to 360° without fracturing or cracking, but they crack and splinter easily when pressure is applied on the (100) face. As observed by scanning electron microscopy (SEM), unlike the smooth monolithic appearance of the straight part (Figs. 1(a,b)), the bent part of the crystal is striated and features distinctive layers, which are separated from each other with an average distance of 3.16 μm (Fig. 1(c)). AFM images of the (001) face reveal a staircase-like topography, while the (100) face is undulated and ridged, where each ridge has a fine structure (Fig. 1(d)), supporting the delamination mechanism. Upon bending, the crystal retains its macroscopic integrity by increasing the mosaic spread by sliding its layers atop each other, which becomes possible through regenerative Cl $\cdots$ Cl bond (interaction) breaking and reformation.

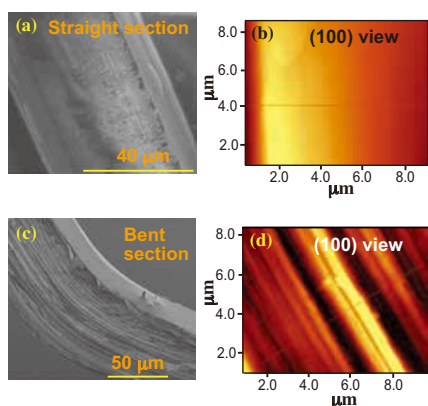


Fig. 1. Surface morphology of the (001) face of a bent HCB crystal at the straight and bent sections, observed by SEM and AFM. Note that the layers appearing in the bent location are absent in the straight location.

To provide insight into the changes that occur during delamination at the molecular and supramolecular levels, we carried out non-polarized and linearly polarized μ -focus IR spectroscopy (BL43IR) and μ -focus X-ray analysis (BL40XU) with synchrotron radiation available at SPRING-8. The IR spectra of a mechanically bent HCB crystal were recorded at a resolution of 2 cm^{-1} on the concave (cc) and convex (cx) sides in the bent region. These spectra were compared to the straight sections of the crystal (Figs. 2(a,c)). The straight part of the crystal reproduced the spectra of an unbent crystal, but, the bands in the bent region were considerably broader. Shifts and splitting were also observed, supporting the increased mosaic spread in the bent region.

We presumed that if bending is due to sliding of the layers which causes the interlayer Cl $\cdots$ Cl bond (interaction) rupture and reformation, then it should be reflected in the stretching frequency of the C—Cl bonds (at $\nu_{\text{C-Cl}} \sim 700 \text{ cm}^{-1}$), as well as in the C=C bonds along the bent region. In fact, both polarized and non-polarized spectra on the convex (cx) side exhibited a blue-shifted $\nu_{\text{C-Cl}}$ band compared to the concave (cc) side, indicating strengthening of the C—Cl bonds and weakening of the Cl $\cdots$ Cl interactions in the cx region. The broadening and splitting of the intrinsic $\nu_{\text{C=C}}$ modes at 1345 and 1295 cm^{-1} , which are expected to be less sensitive to the intermolecular interactions relative to the $\nu_{\text{C-Cl}}$ modes, indicate a reduced symmetry of the HCB molecules upon bending (Fig. 2(c)). The decreased intensities of the Raman bands at 332 cm^{-1} (in-plane $\delta(\text{C-Cl})$) and 229 cm^{-1} (out-of-plane $\delta(\text{C-Cl})$) are in line with the weakening of the Cl $\cdots$ Cl interactions at the cx side of the bent crystal.

To understand the structural mechanism for bending the HCB crystal, we performed spatially resolved X-ray diffraction analysis with a microfocus X-ray setup using synchrotron radiation. A moderately bent crystal was scanned across the bent locations at ten equidistant points (Fig. 2(b)). Small but significant perturbations of the unit cell parameters were observed, supporting the fact that the slippage of layers is associated with expansion or contraction of the crystal lattice. As shown in Figs. 2(d-g), the cell axes (a , b , and c) of the lattice are slightly expanded on both the cx and cc sides, while the angle β increases from the cx to cc side. A closer look at the packing structure of HCB indicates that the Cl $\cdots$ Cl intermolecular interactions are roughly oriented on the ac plane while the $\pi\cdots\pi$ -stacking interactions are oriented along the b axis. Thus, elongations of the a and c axes in both the cx and cc regions are related to the

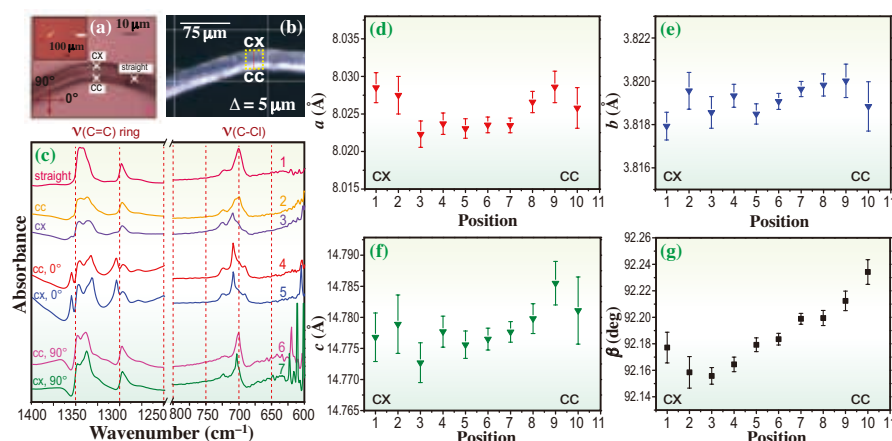


Fig. 2. Unpolarized and polarized IR spectra (a, c) and perturbed unit cell parameters (d-g) of a moderately bent HCB crystal measured across the crystal kink (b).

increased mosaicity mediated by reorganization of the Cl...Cl interactions and splaying of the molecules in the layers, leading to changes in the b axis. This structural adaptation of the crystal lattice under external force prevents the crystal from rupturing.

We postulated that the sliding of layers due to the applied tensile force leads to changes in the crystal density as well as the mechanical response along the bent and unbent locations of the crystal. In fact, our hot-stage experiment reveals that the bent part of the crystal melts earlier than the straight part, which is consistent with a lower density at the bent location of the crystal. In order to study the nanomechanical properties of a bent slender crystal of HCB, exhaustive nanoindentation measurements were performed by profiling the crystal across different faces in the bent and unbent sectors. A clear trend of decreasing elastic modulus (E) and

hardness (H) is observed when going from the straight to the bent location, which is a signature of the softer and more ductile nature of the bent part due to increased the mosaic spread upon sliding of the layers.

Based on the above analysis, we present a model (Fig. 3) for the mechanism of plastic bending of HCB crystals. Applying three-point local pressure on the (001) face results in the formation of ridged layers, which glide atop each other by bond breaking and reformation of the interlayer Cl...Cl bonds (interactions) accompanied with unit cell perturbation, where the cell axes are stretched on the cx side and the cc side. The overall crystal integrity is preserved due to this structural adaptation at the molecular level as well as at the supramolecular level. We postulate that the mechanism proposed here for HCB may be used to explain the extraordinary plasticity of other bendable crystals.

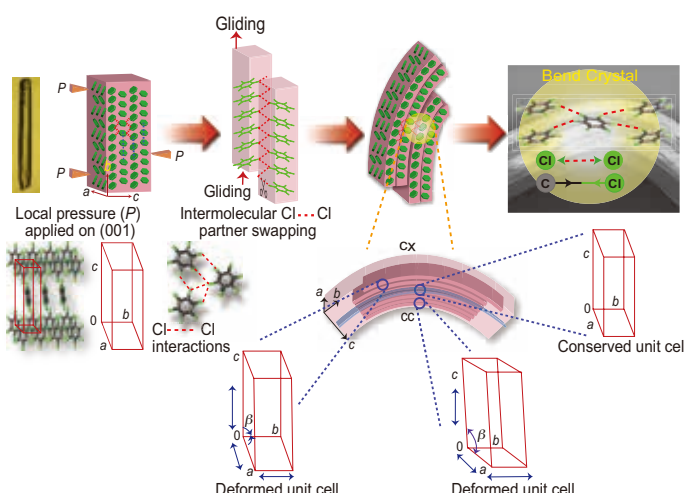


Fig. 3. Model for the mechanism of plastic bending of HCB crystal. Applying external pressure (P) on the (001) face causes gliding of the slip layers by weakening of the interlayer Cl...Cl interaction followed by regenerative partner swapping of the Cl...Cl interaction, which makes bending possible without losing the crystal integrity. Unit cell at convex (cx) and concave (cc) positions appear to be deformed due to stretching and gliding of the layers.

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References

- [1] S. Kobatake *et al.*: Nature **446** (2007) 778.
- [2] C.M. Reddy *et al.*: Chem. Eur. J. **12** (2006) 2222.
- [3] C.M. Reddy *et al.*: Cryst. Eng. Comm. **12** (2010) 2296.
- [4] M.K. Panda, T. Runčevski, S.C. Sahoo, A. Belik, N.K. Nath, R.E. Dinnebier, P. Naumov: Nat. Commun. **5** (2014) 4811.
- [5] M.K. Panda, S. Ghosh, N. Yasuda, T. Moriwaki, G.D. Mukherjee, C.M. Reddy, P. Naumov: Nat. Chem. **7** (2015) 65.

Highly efficient carbon monoxide trap by a transformable soft nanospace of porous crystal

Carbon monoxide (CO) is a central resource for carbon-based chemical products. Although industrial processes such as steel manufacturing produced vast quantities of CO in a mixed exhaust gas, most of it cannot be used as a carbon resource. Therefore, separating CO from a gas mixture has economic and environmental importance. To date, CO separation has been limited to processes using the strong chemisorption of CO, but a high temperature is required to release CO, which is energy consuming. The key to overcoming this problem is a synergistic system that uses a weak local interaction and a subsequent global structural change. This process has been achieved in the nanometer-sized space of a soft biosystem. Despite their crystalline form, porous coordination polymers (PCPs) can provide a nanometer-sized soft space, which can transform in response to guest accommodation or physical stimuli [1–4]; this feature has encouraged us to create a synergistic system in a PCP solid to achieve the ultimate separation of mixed gases. Herein we report a new PCP that has specific but weak CO adsorption/recognition sites and a reversibly transformable framework [5].

We prepared PCP **1** $[\text{Cu}(\text{aip})(\text{H}_2\text{O})](\text{solvent})_n$, which is composed of 5-azidoisophthalate (aip) and divalent Cu^{2+} ions. In the as-synthesized crystal of PCP **1**, the Cu^{2+} and aip ligands form paddle-wheel-type dimeric Cu^{2+} clusters, forming an infinite kagomé-type two-dimensional (2D) sheet structure. The 2D sheets are stacked, creating two types of one-dimensional (1D) infinite channels with cross-section sizes of 9×9 (channel *L*; hexagonal larger channel) and 4×4 (channel *S*; triangular smaller channel) $\text{\AA}^2$ (Fig. 1). Guest molecules are easily removed by heating to give a dried PCP **2** $[\text{Cu}(\text{aip})]_n$.

The crystal structure of dried PCP **2** was determined by Rietveld analysis using synchrotron powder X-ray diffraction data obtained at **BL44B2** beamline. Drying induces marked changes in the channel *S* shape;

channel *S* in dried PCP **2**, is squeezed at the neck, almost creating separate pores. This separation should greatly hinder the access of guest molecules.

The most competitive gas to CO is nitrogen (N_2), which is present in large amounts in industrial exhaust gas as an impurity. Typically it is difficult to distinguish and separate CO and N_2 in industrial processes due to their similar electronic structures, sizes, and physicochemical properties. After deducing that PCP **2** shows a contrasting sorption behavior for these two gases, we studied the sorption behaviors of CO and N_2 on PCP **2** and the structural changes upon sorption by *in situ* X-ray powder diffraction (XRPD)/sorption coincident measurements (Fig. 2). During the N_2 adsorption process, no appreciable positional changes in the peaks of the XRPD patterns were observed. However, pronounced changes in the XRPD patterns were observed upon CO adsorption and desorption. Although the XRPD pattern of PCP **2** did not change markedly in the initial CO adsorption, after point c in Figs. 2C and 2D, the original peaks of PCP **2** gradually decreased and new peaks appeared. These results clearly show that the steep uptake after point c was accompanied by a structural change. In addition, we successfully obtained the crystal structure of PCP **3** containing a Cu^{2+} –CO complex module. Structural analysis revealed that the coordination of CO induced a global structural change and created a suitable compartment for further accommodation of CO molecules in the center of channel *S*.

The mechanism for the stepwise sorption of CO can be explained as follows (Fig. 3). In the first step of the isotherm, CO molecules are physically and exclusively absorbed in the larger channel *L* without marked structural change to the framework due to poor accessibility of the highly constricted channel *S*. After filling channel *L*, CO molecules start to form Cu^{2+} –CO bonds, inducing a global structural transformation that expands the squeezed paths. This promotes additional CO adsorption in the center of channel *S*, which is the so-called self-accelerating gas adsorption. The adsorption event in channel *S* is reminiscent of a biosystem where a heme-protein efficiently traps oxygen molecules with a structural change, which is known as an allosteric effect.

We tested its CO separation ability using CO and N_2 mixtures with various compositions. Exposing dried PCP **2** to mixtures of CO and N_2 at 100 kPa, results in an equilibrium state upon gradually decreasing the temperature from 300 to 81 K. The adsorbed gas ratios were examined by gas chromatography. For example,

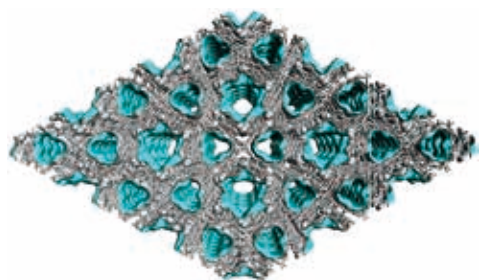


Fig. 1. Crystal structure of PCP **1**. Guest molecules are omitted for clarity.

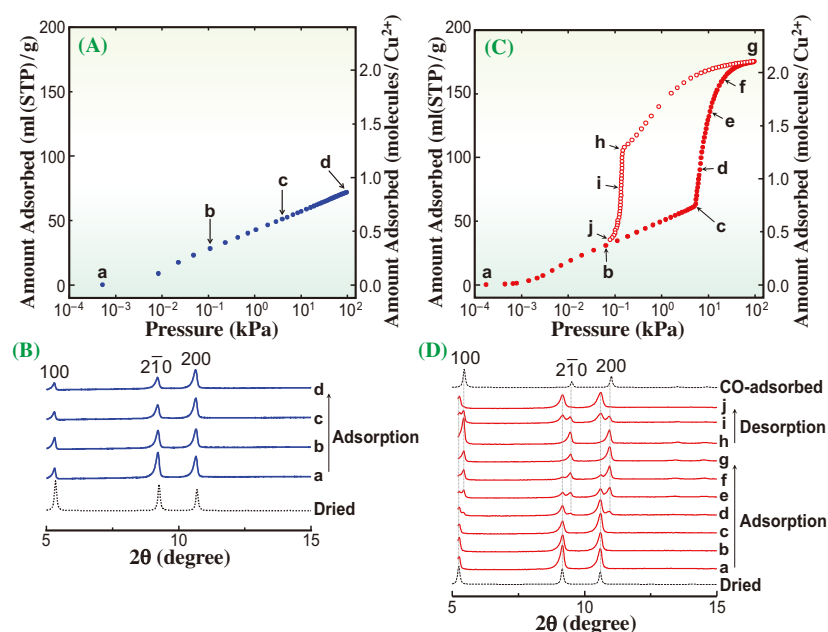


Fig. 2. Coincident XRPD/sorption measurements of PCP 2. (A) N_2 sorption isotherm at 120 K. (B) XRPD patterns measured at each point shown in the N_2 sorption isotherms. (C) CO sorption isotherms at 120 K. (D) XRPD patterns measured at each point in the CO sorption isotherms.

a surprising enrichment of CO of 94% was achieved after the second cycles starting from a 1:1 mixture of CO and N_2 . Because of the size restrictions, neither CO nor N_2 can diffuse into channel S without expansion of the narrow path. However, CO can expand the path and realize CO diffusion into the channel when it breaks the coordination bond between Cu and O and subsequently forms a coordination bond with Cu. On the other hand, the weak coordination ability prevents N_2 from expanding the path. Even after CO expands the path, N_2 cannot diffuse into the channel because the paths are surrounded by the three coordinated CO molecules, which sterically inhibit N_2 .

In conclusion, our newly synthesized nanoporous crystalline material can realize a highly effective separation of CO from a competitive gas mixture. The self-accelerating sorption system, which is triggered by a weak coordination interaction, is reminiscent of that in soft biological systems such as hemoglobin. In this sense, our artificial soft porous crystalline is the first system to exhibit a highly efficient molecular trapping and releasing ability similar to a biological system. We believe that this new concept of “self-accelerating sorption in a soft nanoporous crystal” will lead to a new approach to separate and store valuable gases without consuming large amounts of energy.

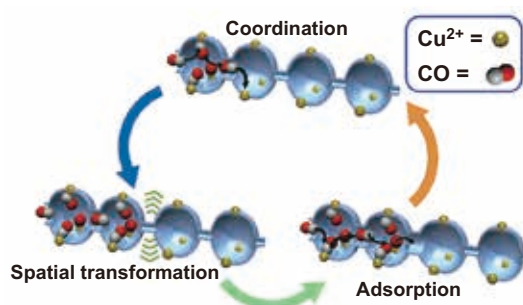


Fig. 3. Schematic representation of the self-accelerating CO adsorption mechanism in PCP 2.

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References

- [1] S. Shimomura *et al.*: Nat. Chem. **2** (2010) 633.
- [3] H. Sato *et al.*: Nat. Mater. **9** (2010) 661.
- [4] S. Kitagawa and R. Matsuda: Coord. Chem. Rev. **251** (2007) 2490.
- [5] H. Sato, W. Kosaka, R. Matsuda, A. Hori, Y. Hijikata, R.V. Belosludov, S. Sakaki, M. Takata, S. Kitagawa: Science **343** (2014) 167.

Acute and obtuse rhombohedrons in the local structures of relaxor ferroelectric $\text{Pb}(\text{Mg}_{1/3}\text{Nb}_{2/3})\text{O}_3$

Nanoscale heterogeneous structures play a crucial role in the appearance of gigantic susceptibilities and the associated novel phenomena in solids. Relaxor ferroelectrics have attracted much attention in the past few decades due to their complex local structures, puzzling mechanisms, and potential applications as high-performance electromechanical transducers based upon their excellent piezoelectric properties. However, because the nanoscopic heterogeneity inherent to relaxors creates challenges, the microscopic origin of the outstanding piezoelectric properties of relaxor-based ferroelectrics is uncertain.

X-ray fluorescence holography (XFH) is a model-free bulk sensitive technique to determine the local structure, enabling the 3D atomic environment around a selected element to be visualized within a radius of a few nanometers at an atomic resolution. These features make XFH method ideally suitable to investigate nanoscale heterogeneous structures in solids [1]. In this work, we investigate the local-three-dimensional (3D) atomic arrangements around the Pb^{2+} and Nb^{5+} ions in a prototypical relaxor [2], ferroelectric $\text{Pb}(\text{Mg}_{1/3}\text{Nb}_{2/3})\text{O}_3$ (PMN) [3,4], using XFH.

The experiments were carried out at beamline BL22XU. XFH data were recorded at incident X-ray energies between 19.0 - 23.5 keV in 0.25 keV steps. Figure 1(a) shows the details of the experimental setup. Using a cylindrical graphite-crystal energy analyzer, Nb $K\alpha$ (16.61 keV) and Pb $L\alpha$ (10.55 keV) fluorescent X-rays from the sample were analyzed and focused onto an avalanche photodiode (APD), respectively. The measurement time for one energy hologram was about 3 h. Figure 1(c) shows a measured Nb hologram pattern taken at 20.0 keV. The hologram data were symmetrized using the average $Pm\bar{3}m$ cubic symmetry of the sample (Fig. 1(b)) and the X-ray standing wave lines, and then extended to the 4π sphere as shown in Fig. 1(d). The 3D real-space atomic images around Nb and Pb were reconstructed from the multi-energy holograms using Barton's multiple energy algorithm [5].

Figure 2(a) shows the reconstructed atomic images on the (110) plane through the emitter Nb. Pb atomic images are clearly visible at the ideal positions, and obviously elongated along the radial direction. All of the Nb/Mg atoms on the B sites are hardly observed, indicating that the Nb/Mg atoms are largely disordered because the image intensity of an atom is reduced by its fluctuations. The Pb atoms are visible around the emitter Nb due to the atomic correlation between the Pb and Nb atoms. Only the nearest Pb atoms exhibit

$\langle 111 \rangle$ correlations with Nb. Figure 2(b) shows a fine 3D image of the first neighboring Pb atoms at the $\frac{111}{222}$ positions. The cube indicates 1/8 of the unit cell of PMN. Surprisingly, four remarkable images are visible along the $\langle 111 \rangle$ direction, which are labeled 1-4 Fig. 2(b). The interatomic distances between the central Nb and these four Pb images are 2.73 Å, 3.21 Å, 3.71 Å, and 4.25 Å. The Pb1 and Pb4 images show a center symmetry with respect to the ideal position (3.5 Å).

Next, atomic images around the emitter Pb were reconstructed using the Pb $L\alpha$ holograms to evaluate the Pb-Pb correlation. Since only the 200, 220, and 111 atomic images are visible in Figs. 2(c) and 2(d), a body center-like $2a_0 \times 2a_0 \times 2a_0$ superlattice structure emerges ($a_0 = 4.05$ Å). The Pb atoms around the emitter Nb are more visible (Fig. 2(a)), suggesting that the stability of the Pb atoms is relatively lower than the Nb atoms.

The rhombohedral distortion explains well the separation of the Pb images in Fig. 2(b), and our calculations show that the rhombohedral deformation of the perovskite cube with rhombohedral angles of 77.0° and 100.6° give rise to two Pb-Nb interatomic distance pairs of 3.23 and 4.22 Å, and 2.78 and 3.71 Å, respectively. These values are close to the experimentally observed ones, confirming that there are acute and obtuse rhombohedrons in the local

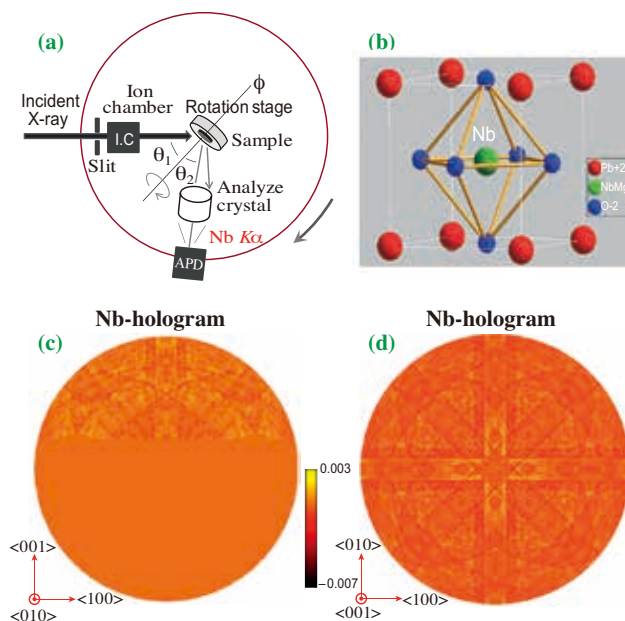


Fig. 1. (a) XFH experimental setup. (b) Ideal structure of perovskite PMN. (c) Nb hologram of PMN on a spherical surface. Lower areas indicate the part of the full holographic information that could not be measured. (d) Extended 4π hologram of Nb hologram in (c).

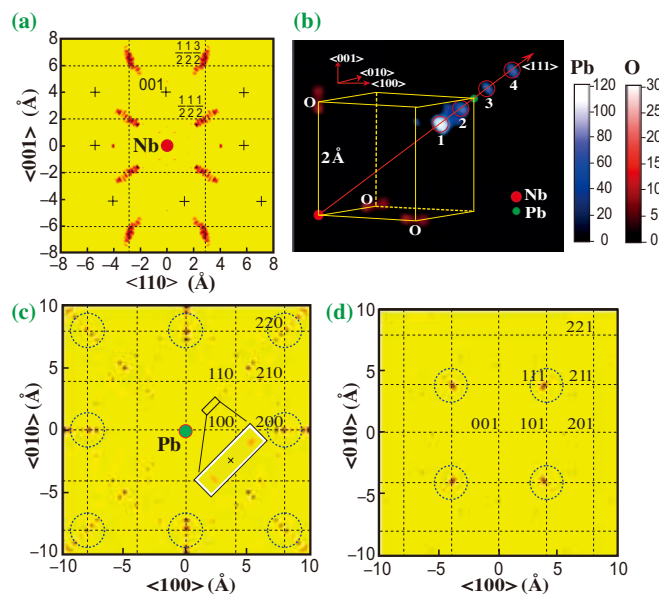


Fig. 2. Atomic images in PMN. (a) (110) plane around the emitter Nb. Intersections of dotted lines indicate the ideal atomic positions of Pb atoms in (a), (c), and (d). Crosses in (a) indicate the ideal positions of Nb/Mg. (b) 3D images of the nearest Pb atoms around Nb. Atomic images around the emitter Pb on the (001) plane at (c) $z = 0$ Å and (d) $z = 4$ Å.

structure of PMN (Fig. 3(a)).

To understand the Pb2 and Pb3 images in Fig. 2(b), we introduced a disk-like 2D Gaussian distribution with a mean-square displacement (σ_a) and calculated the image intensities of the Pb2 and Pb3 atoms by changing the value of σ_a . The calculated atomic images with $\sigma_a = 0.19$ Å agree well with the experimental data, which indicates that there are two distorted rhombohedrons in PMN for the Pb-Nb correlations and a nanoscale antidistortive transformation. Combining these two rhombohedral unite cells (Fig. 3(c)), constructs a body-center-like 3D network structure, which is comprised of positionally

stable Pb ions and off-centered Pb and Nb ions fluctuating around their corresponding ideal perovskite position. The estimated size of the corresponding domain is 1-2 nm suggesting that the fluctuating Pb and Nb ions are responsible for the characteristic broad relaxation spectrum observed in PMN.

The present work demonstrated the new potential of XFH to characterize disordered systems and to provide more detailed structural information. This capability is a powerful tool in understanding the relations between the properties and the local structures in disordered functional materials.

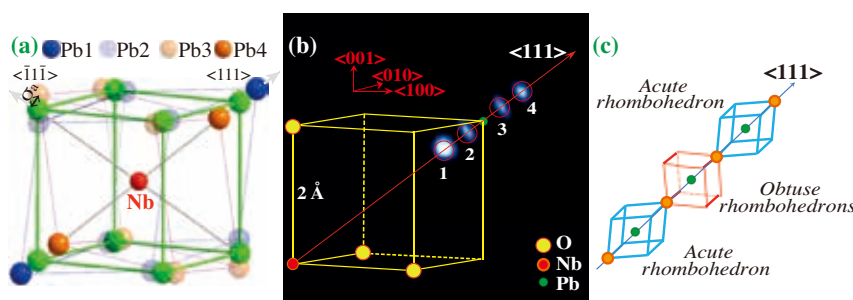


Fig. 3. Reproduction of the nearest Pb images around Nb, based on a rhombohedral transformation model of the unit cells. (a) Schematic of transformation of the unit cell from cubic to acute and obtuse rhombohedrons. σ_a is the FWHM of the 2D Gaussian distribution of Pb2 and Pb3 parallel to the (111) plane. (b) 3D images of the nearest Pb atom around Nb reconstructed from the calculated holograms with $\sigma_a = 0.19$ Å. (c) 3D network of acute and obtuse rhombohedrons.

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References

- [1] K. Hayashi *et al.*: J. Phys.: Condens. Matter **24** (2012) 093201.
- [2] W. Hu, K. Hayashi, K. Ohwada, J. Chen, N. Happo, S. Hosokawa, M. Takahashi, A.A. Bokov and Z.-G. Ye: Phys. Rev. B **80** (2014) 140103(R).
- [3] G.A. Smolenskii and A.I. Agranovskaya: Sov. Phys. Tech. Phys. **2** (1961) 2584.
- [4] A.A. Bokov and Z.G. Ye: J. Mater. Sci. **41** (2006) 31.
- [5] J.J. Barton: Phys. Rev. Lett. **67** (1991) 3106.

Lattice symmetry breaking at the hidden-order transition in URu₂Si₂

To elucidate the nature of a phase transition in materials, the most important step is to identify which symmetries are broken below the transition temperature. In 1985, a large anomaly in the specific heat was observed in the heavy-fermion metal, URu₂Si₂, at 17.5 K, indicating the presence of a phase transition at this temperature. Since then, tremendous efforts have been made to study the nature of this transition, but neither a symmetry change in the crystal structure nor a large magnetic moment that can account for the entropy change has been observed. This enigmatic order is thus called the “hidden order,” and understanding this hidden-order transition is a long-standing issue in condensed matter physics [1].

Recently, magnetic torque measurements in small pure crystals under an in-plane magnetic field rotation have shown that the two-fold oscillation as a function of field angle in the *ab* plane starts to develop below the hidden-order transition temperature (T_{HO}). This two-fold oscillation indicates that the four-fold rotational symmetry associated with the body-centered tetragonal crystal structure (Fig. 1(a)) is broken in the

hidden-order phase [2]. Similarly, cyclotron resonance [3] and nuclear magnetic resonance experiments [4] under an in-plane field rotation have provided evidence for four-fold rotational symmetry breaking below T_{HO} . However, these experiments were conducted under an in-plane magnetic field, which itself can break the rotational symmetry. Thus, direct observation of symmetry breaking in the absence of a magnetic field is required to identify the ground state of the hidden order.

To this end, we performed high-resolution synchrotron X-ray crystal structure analysis of URu₂Si₂ at beamline BL02B1 [5]. We used ultraclean single crystals with very high residual resistivity ratios of ~ 670 , which have become available recently [3]. First, out of more than ~ 30 crystals, we selected a high crystalline quality sample with the sharpest high-angle Bragg peak, which was measured using an imaging plate at room temperature. Then, we tuned the X-ray energy to 17.15 keV, which is just below the absorption edge of uranium so that the X-ray attenuation length is sufficient to obtain bulk information. To realize a high

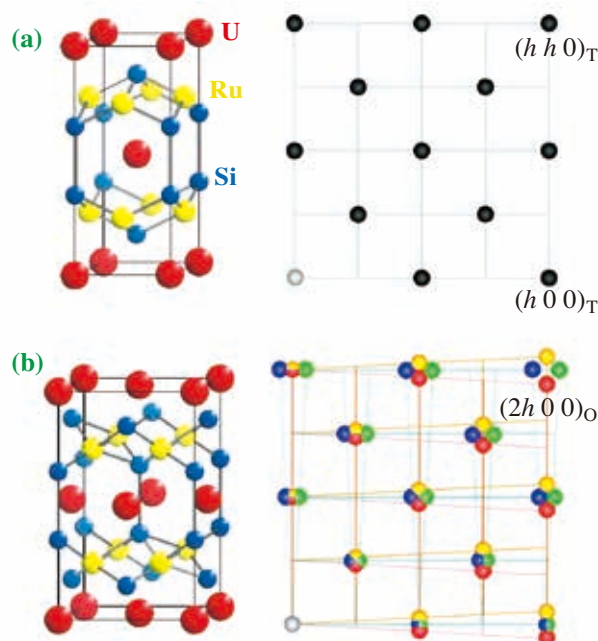


Fig. 1. (a) Crystal structure of URu₂Si₂ above the hidden-order transition temperature T_{HO} , which is $I4/mmm$ body-centered tetragonal type. Schematic Bragg spots (black circles) in the $(h k 0)$ plane ($h, k \geq 0$) are also shown on the right. Left bottom is the origin. (b) Orthorhombic $Fmmm$ structure in the ordered phase below T_{HO} identified in our study. Bragg spots split due to formation of the domains. Colored circles correspond to the four different domains.

resolution, we used a high-angle reflection set-up in which the four-circle diffractometer was equipped with a cryocooler. We focused on the high-angle $(880)_T$ Bragg peak at a reflection angle 2θ above 165 degrees, which corresponds to the experimental resolution of a lattice constant as good as 3×10^{-5} .

Figure 2(a) shows the temperature dependence of the $(880)_T$ Bragg peak measured by the $2\theta/\theta$ scattering mode. Below the hidden-order transition at $T_{HO} = 17.5$ K, clear peak splitting is observed, indicating that symmetry-breaking lattice distortion sets in just below the transition. We also performed two-dimensional scans in the $(hk0)$ plane as shown in Figs. 2(b) and 2(c). The single peak at 19 K (above T_{HO}) clearly splits at 10 K (below T_{HO}). The integrated intensities of these data are identical within experimental error. Our analysis indicates that the profile at 10 K is consistent with the four-fold splitting of the single Bragg peak, as expected for the orthorhombic $Fmmm$ -type crystal

structure shown in Fig. 1(b) [5]. Hence, we conclude that the space symmetry of the hidden order belongs to this orthorhombic type, which breaks four-fold rotational symmetry. Our observation is fully consistent with previous high-field measurements [2-4], and indicates that rotational symmetry breaking is not field induced but is an intrinsic property of the hidden order.

To our knowledge, this is the first direct observation of a symmetry change in the hidden-order phase transition in URu_2Si_2 by scattering experiments. The clarified space symmetry places very strong constraints on the genuine hidden order parameter. Thus, the present results can be regarded as a big step toward the full resolution of this 30-year old mystery. In addition, we believe that understanding the origin of such spontaneous rotational symmetry breaking found in URu_2Si_2 may be important to uncover the nature of other unusual states of matter hidden in several strongly correlated electron systems.

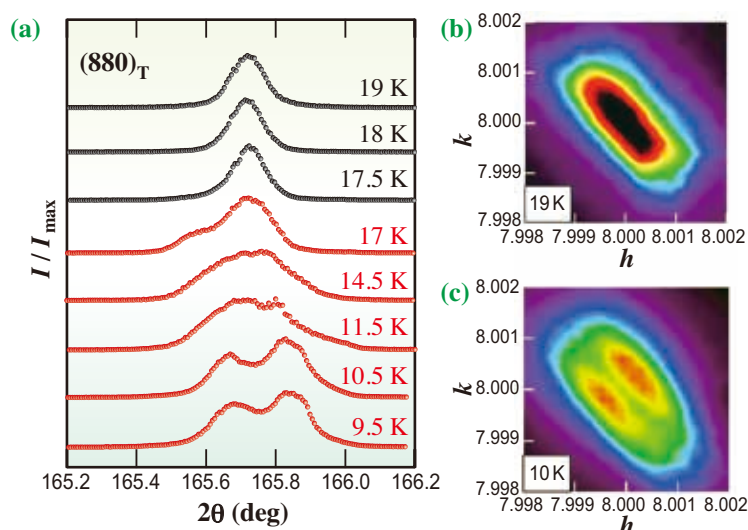


Fig. 2. (a) Temperature dependence of the Bragg peak $(880)_T$ in an ultraclean single crystal of URu_2Si_2 . Each curve is shifted vertically for clarity. Below the hidden order transition at $T_{HO} = 17.5$ K, clear splitting of the Bragg peak is observed. (b) Two-dimensional intensity plot in the $(hk0)$ plane near the $(880)_T$ Bragg peak at 19 K (above T_{HO}). (c) Similar data for 10 K (below T_{HO}).

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References

- [1] See, for a review, J.A. Mydosh and P.M. Oppeneer: Rev. Mod. Phys. **83** (2011) 1301.
- [2] R. Okazaki *et al.*: Science **331** (2011) 439.
- [3] S. Tonegawa *et al.*: Phys. Rev. Lett. **109** (2012) 036401.
- [4] S. Kambe *et al.*: Phys. Rev. Lett. **110** (2013) 246406.
- [5] S. Tonegawa, S. Kasahara, T. Fukuda, K. Sugimoto, N. Yasuda, Y. Tsuruhara, D. Watanabe, Y. Mizukami, Y. Haga, T.D. Matsuda, E. Yamamoto, Y. Onuki, H. Ikeda, Y. Matsuda and T. Shibauchi: Nat. Commun. **5** (2014) 4188.

Electron-doping evolution of spin and charge excitations in cuprate superconductors

Superconductivity, which is a state of matter where the electrical resistance disappears completely, is a fascinating phenomenon for many researchers in condensed matter physics and materials science. In particular, high- T_c superconductivity of cuprates has attracted great interest since its initial discovery more than a quarter century ago.

The parent compound of cuprate superconductors is an antiferromagnetic Mott insulator, where electrons cannot move in the crystal due to their strong Coulomb repulsion. Upon doping either the electrons or holes as mobile charge carriers, the compound becomes metallic and the antiferromagnetic spin order fluctuates. Superconductivity occurs in the doped metallic state. Additionally, the antiferromagnetic interaction, which causes the spin order in the parent compound, is intimately related to the superconductivity. Such a complicated spin and charge behavior is a characteristic of strongly correlated electrons. Both spin and charge excitations should be clarified for a definitive understanding of the electron dynamics in cuprates, and the differences or similarities between electron- and hole-doped compounds have been a central issue in the study of cuprate superconductors.

In this study [1], we used resonant inelastic X-ray scattering (RIXS) to measure the spin and charge excitations in the electron-doped superconductor, $\text{Nd}_{2-x}\text{Ce}_x\text{CuO}_4$. Very recently, RIXS at the transition-metal L -edge became a viable alternative to inelastic neutron scattering (INS) to measure momentum-resolved spin excitations. On the other hand, charge excitations can be observed at both the L - and K -edges. Our RIXS experiments at the $\text{Cu } L_3$ -edge and K -edge were performed at ID08 of the ESRF and

BL11XU of SPring-8, respectively. In the principal publication, we also measured the INS spectra using the chopper spectrometer, 4SEASONS at J-PARC.

Figure 1(a) shows the $\text{Cu } L_3$ -edge RIXS spectra of the parent Nd_2CuO_4 ($x = 0$) at three momenta in the CuO_2 plane ($\mathbf{q}_{\parallel}$). The spectra can be univocally decomposed into the elastic (green), single spin-flip (single-magnon, blue), multiple spin-flip (multi-magnon, yellow), and dd (local electron transition between the $\text{Cu } 3d$ orbitals, gray) components. The single-magnon is the dominant component. In the electron-doped superconductor, $\text{Nd}_{1.82}\text{Ce}_{0.18}\text{CuO}_4$ ($x = 0.18$) shown in Fig. 1(b), the single-magnon component is still dominant, but additional spectral weight (red) appears at $\mathbf{q}_{\parallel} = (0.09, 0)$ and $(0.18, 0)$. Unlike the almost dispersionless multi-magnon in the parent compound, the additional weight has a steep dispersion. Figures 2(a) and 2(b) show the $\text{Cu } L_3$ -edge RIXS intensity maps of $x = 0$ and 0.18, respectively, where the blue squares are the superimposed peak positions of the spin excitations (single-magnon). The spin excitations of $x = 0$ have sinusoidal dispersion, as expected for antiferromagnetic spin-wave excitations. Upon electron doping ($x = 0.18$), the spin excitation moves to a higher energy and is accompanied by a broadening of the width. We also confirmed the high-energy shift of the spin excitations near the magnetic zone center by INS. This doping evolution of the spin excitations is in distinct contrast of the hole-doped case, where the spectral distribution of the spin excitations broadens but its energy position is almost unchanged [2].

Figure 1(c) shows the $\text{Cu } K$ -edge RIXS spectra of $x = 0.18$. We observed two types of charge excitations: interband excitations across the charge-transfer gap

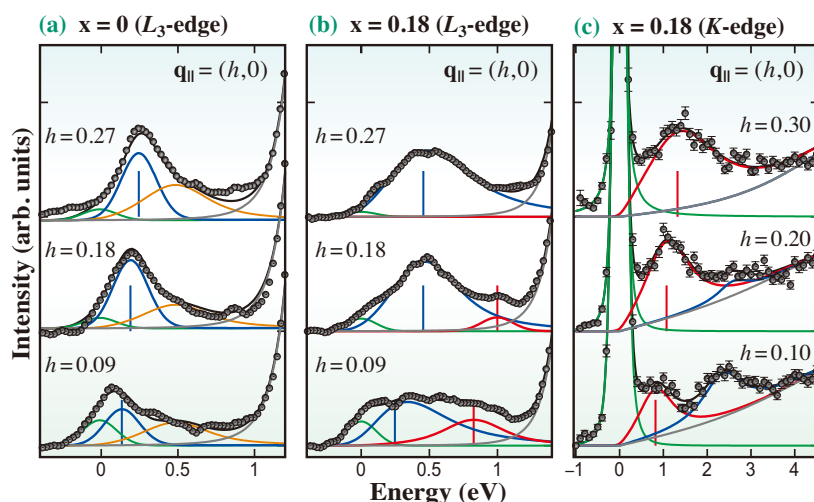


Fig. 1. RIXS spectra of $\text{Nd}_{2-x}\text{Ce}_x\text{CuO}_4$. Solid circles and solid lines denote the experimental data and the fitting results, respectively. Blue and red vertical bars indicate the peak positions of spin excitations (single-magnon) and intraband charge excitations, respectively.

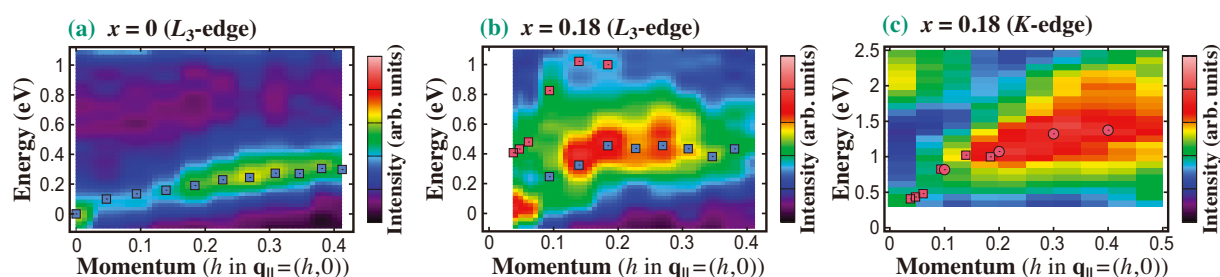


Fig. 2. RIXS intensity map of $\text{Nd}_{2-x}\text{Ce}_x\text{CuO}_4$. Blue and red squares indicate the peak positions of the spin and charge excitations obtained by fitting the Cu L_3 -edge RIXS spectra, respectively. Red circles are the peak positions of the intraband charge excitations in the Cu K -edge RIXS spectra.

(light blue), which are prominent near the Brillouin zone center, and dispersive intraband excitations in the upper Hubbard band (red). In the parent Mott insulator, the latter is missing and charge excitations appear above 2 eV. The momentum dependence of the two excitations is clear in the RIXS intensity map in Fig. 2(c) and is consistent with our previous work for $x = 0.15$ [3]. The red circles on the map are the peak positions of the intraband excitations. Due to the improved energy resolution compared to the previous work, the lower- q region, could be accessed, allowing the additional spectral weight to be identified in the Cu L_3 -edge RIXS spectra. We also plotted the peak of the additional spectral weight in the map (red squares). It is clear that the peak positions are smoothly connected to the dispersion of the intraband excitations in the Cu K -edge RIXS. Thus, the additional spectral weight in the Cu L_3 -edge RIXS is attributed to the same charge origin. The energy scale of the charge excitations is on the order of transfer energy ($t \sim 0.4$ eV), and should be higher than the spin excitations, which is on energy scale of the exchange interaction ($J \sim 0.1$ eV). It is consistent with our assignment in the Cu L_3 -edge RIXS spectra.

From RIXS at the Cu L_3 - and K -edges, which is complemented by INS in the principal publication, we clarified the electron-doping evolution of the spin and charge excitations in cuprate superconductors. Figure 3, schematically summarizes the doping evolution of the spin and charge excitations, where characteristics of the elementary excitations in the cuprate superconductors are displayed. The high-energy shift of the spin excitations and their mixture with the charge excitations indicate that the electron dynamics of the electron-doped cuprates has a highly itinerant character in the sub-eV energy scale. In contrast, dispersion of the spin excitation in the hole-doped cuprates follows the spin-wave excitations of the parent antiferromagnetic insulator, which means that a more localized picture is suitable. Our findings impose constraints on theoretical models and show that a comprehensive description of the electronic excitations in the electron- and

hole-doped cuprates is a prerequisite to completely understand the superconductivity. For instance, a recent theoretical study demonstrated that the t - J model, which is often used to describe the electronic properties of copper oxides, does not reproduce the high-energy shift of the spin excitations observed in this study [4]. Incidentally, our work is the first to demonstrate that the complementary use of X-rays and neutrons is very effective in inelastic scattering.

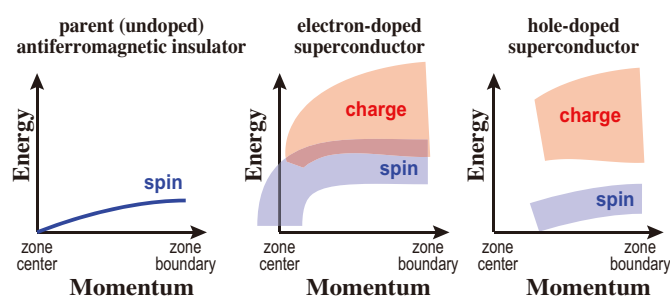


Fig. 3. Doping evolution of spin and charge excitations in the cuprate superconductors. In the present work, the parent antiferromagnetic insulator and an electron-doped superconductor are studied. Excitations in hole-doped superconductors have been reported in Refs. 2,5.

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References

- [1] K. Ishii, M. Fujita, T. Sasaki, M. Minola, G. Dellea, C. Mazzoli, K. Kummer, G. Ghiringhelli, L. Braicovich, T. Tohyama, K. Tsutsumi, K. Sato, R. Kajimoto, K. Ikeuchi, K. Yamada, M. Yoshida, M. Kurooka and J. Mizuki: *Nat. Commun.* **5** (2014) 3714.
- [2] M. Le Tacon *et al.*: *Nat. Phys.* **7** (2011) 725.
- [3] K. Ishii *et al.*: *Phys. Rev. Lett.* **94** (2005) 207003.
- [4] C.J. Jia *et al.*: *Nat. Commun.* **5** (2014) 3314.
- [5] S. Wakimoto *et al.*: *Phys. Rev. B* **87** (2013) 104511.

A complete high-to-low spin state transition of trivalent cobalt ion in octahedral symmetry in $\text{SrCo}_{0.5}\text{Ru}_{0.5}\text{O}_{3-\delta}$

The largest spin state variability of Co^{3+} is reported mainly for metal oxide compound, often within crystal structures where cobalt is coordinated by four to six oxygen ions. Low-spin (LS) Co^{3+} is found in LiCoO_2 , $\text{La}_2\text{Li}_{0.5}\text{Co}_{0.5}\text{O}_4$, and in LaCoO_3 at low temperature. LaCoO_3 is intensively studied and its controversial spin state cross-over upon heating is still debated [1,2]. High-spin (HS) Co^{3+} in a tetrahedral local symmetry was found in a mixed valent cobalt oxide YBaCo_4O_7 . The pure HS Co^{3+} in a square pyramidal CoO_5 coordination occurs in $\text{Sr}_2\text{CoO}_3\text{Cl}$ and BiCoO_3 . However, the pure HS Co^{3+} oxide in octahedral local symmetry is rarely documented. Also, there is a steady debate on the issue whether the octahedrally coordinated Co^{3+} has a mixed HS-LS state or an intermediate spin (IS) state in the new classes of cobaltates $\text{RBaCo}_2\text{O}_{5.5}$ (R =rare-earth metal), $\text{Sr}_{1-x}\text{Y}_x\text{CoO}_{3-\delta}$, $\text{Pr}_{0.5}\text{Ca}_{0.5}\text{CoO}_3$, as well as in LaCoO_3 at high temperature. All the extraordinary material properties of the cobaltates, including superconductivity, giant magnetoresistance, strong thermopower, metal-insulator transitions, as well as spin-blockade behavior are related to the spin state degree of freedom of the cobalt ion. Hence, if the debate around the IS state is settled the interpretations of the material properties of cobalt based metal oxides are simplified remarkably.

The complex metal oxide $\text{SrCo}_{0.5}\text{Ru}_{0.5}\text{O}_{3-\delta}$ possesses a slightly distorted perovskite crystal structure. Figure 1 displays the unit cell of the body centered monoclinic crystal structure as well as the local oxygen coordination of the transition metal site. From the crystal structure refinement, the Co-O distances are unusually large, ranging from 1.96 to 2.00 Å. The Co-K EXAFS data reveal an average Co-O distance of 1.98 ± 0.02 Å [3]. This distance is even larger than that in LaCoO_3 at 1000 K. At 650 K the average distance in LaCoO_3 was found to be 1.949 Å, a state which was proposed to have half of the cobalt ions in a HS state, whereas Co-O distance for IS Co^{4+} ion in SrCoO_3 is 1.918 Å.

Based on soft-X-ray absorption spectroscopy in combination with multiplet calculations, we have demonstrated that the Co^{3+} ion in $\text{SrCo}_{0.5}\text{Ru}_{0.5}\text{O}_{2.96}$ is high-spin, which is unique for Co^{3+} in octahedral oxygen coordination [3]. To investigate whether the HS state in $\text{SrCo}_{0.5}\text{Ru}_{0.5}\text{O}_{2.96}$ can be converted to an IS or LS state on applying pressure, a $\text{K}\beta$ emission experiment was carried out. The high pressure Co $\text{K}\beta$ X-ray emission spectra were obtained at the Taiwan inelastic X-ray scattering beamline

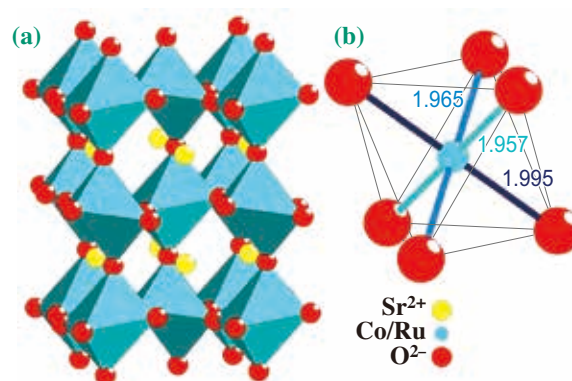


Fig. 1. Part of the $\text{SrCo}_{0.5}\text{Ru}_{0.5}\text{O}_{2.96}$ crystal structure (left) and the local transition metal surrounding with bond lengths in Å to the neighboring oxygen ions (right).

BL12XU. Figure 2(a) shows the evolution of the Co $\text{K}\beta$ X-ray emission line of $\text{SrCo}_{0.5}\text{Ru}_{0.5}\text{O}_{2.96}$ as a function of pressure from ambient up to 39.6 GPa at room temperature. At ambient pressure, the Co $\text{K}\beta$ emission spectrum reveals a main peak located at ~ 7650 eV referred to as the $\text{K}\beta_{1,3}$ line and a pronounced satellite peak located at ~ 7637 eV corresponding to the $\text{K}\beta'$ line. At high pressures, e.g., 39.6 GPa, the satellite peak nearly disappears and the main line gets narrower. This is similar to what happens for the $\text{K}\beta$ emission spectrum of LaCoO_3 at low temperatures, indicating that the LS state has been reached for $\text{SrCo}_{0.5}\text{Ru}_{0.5}\text{O}_{2.96}$. In fact, the spectral changes are alike those found for the complete spin state transition in FeS from 0 to 11.5 GPa and for $\text{Mg}_{0.9}\text{Fe}_{0.1}\text{SiO}_3$ from 20 to 120 GPa, where Fe^{2+} also has the $3d^6$ electronic configuration. This infers that the applied pressure induces a complete HS to LS transition in $\text{SrCo}_{0.5}\text{Ru}_{0.5}\text{O}_{2.96}$. The inset of Fig. 2(a) contains a plot of the so-called integrated absolute difference (IAD) as a function of pressure and the total IAD changes by about 0.15 in going from ambient pressure to 39.6 GPa. This 0.15 value is consistent with what is expected for a complete HS to LS transition.

Important is that the spectral changes with pressure are continuous without any signs for new features that otherwise could indicate the presence of spin states other than the HS and LS. This is apparent in Fig. 2(b), displaying a comparison between the difference spectrum of $\text{K}\beta$ emissions taken at ambient pressure (AP) and at 39.6 GPa (black line) with the difference

spectrum of emissions taken at 15.8 GPa and at 39.6 GPa (green line). The black curve represents the spectral changes associated with a change in the average spin expectation value S from 2 to 0, and the green curve for a change of S from ≈ 1 to 0. Note that the difference spectra are identical apart from the expected scaling factor of two. This infers that the system at 15.8 GPa is in a $\sim 1:1$ mixed HS:LS state, and not in an IS state, as otherwise the difference spectrum would show new features [4].

To understand the above experimental observations, the total energy level diagram of the cobalt ions as a function of Co-O distance was calculated (Fig. 3). Clearly, the ground state of Co^{3+} ions is either HS or LS (not IS) with a crossover at Co-O distance about 1.93 Å, where the HS and LS states are strongly mixed. Thus, Fig. 3 describes the evolution of the spin state during the compression induced by high pressure for $\text{SrCo}_{0.5}\text{Ru}_{0.5}\text{O}_{2.96}$ and reversely, during the expansion upon increasing the temperature for LaCoO_3 . Note that the IS states are remarkably unfavorable in energy in comparison to the HS and LS states.

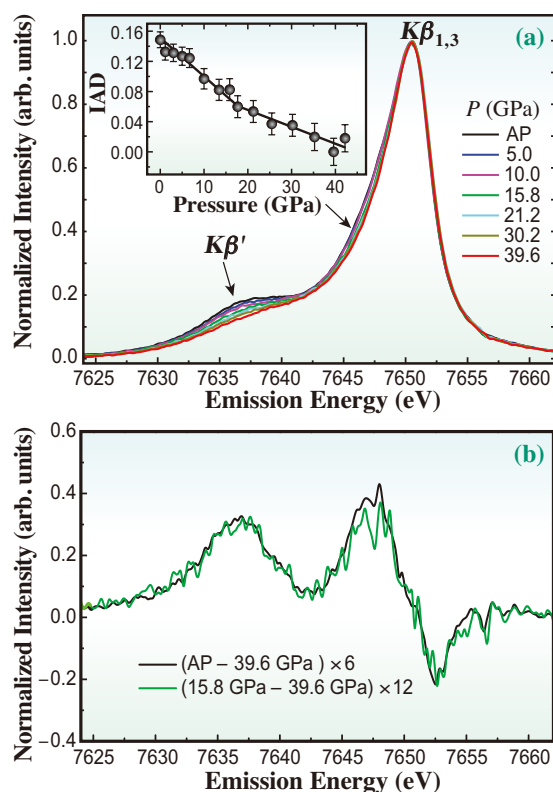


Fig. 2. (a) Evolution of the Co $K\beta$ X-ray emission spectra of $\text{SrCo}_{0.5}\text{Ru}_{0.5}\text{O}_{2.96}$ under pressure up to 39.6 GPa at room-temperature. The inset shows IAD values of Co $K\beta$ emission spectra as a function of applied pressure with respect to 39.6 GPa. (b) Difference spectrum of the Co $K\beta$ emissions taken at ambient pressure (AP) and 39.6 GPa (full black line) and taken at 15.8 GPa and 39.6 GPa (green dashed line).

In fact, the lowest of the IS states is already ~ 0.4 eV (~ 4000 K) higher than the degenerated LS and HS at the Co-O distance 1.932 Å. These results allow us to draw an energy diagram depicting relative stabilities of the high, intermediate, and low spin states as functions of the metal-oxygen bond length for a Co^{3+} ion in an octahedral coordination.

A gradual high-to-low spin state transition in $\text{SrCo}_{0.5}\text{Ru}_{0.5}\text{O}_{2.96}$ is completed by applying high hydrostatic pressure of up to ~ 40 GPa. Across this spin state transition, the Co $K\beta$ emission spectra can be fully explained by a weighted sum of the high-spin and low-spin spectra. We have not found any signs of the much debated intermediate spin state at any stage during the pressure induced spin state transition in this material.

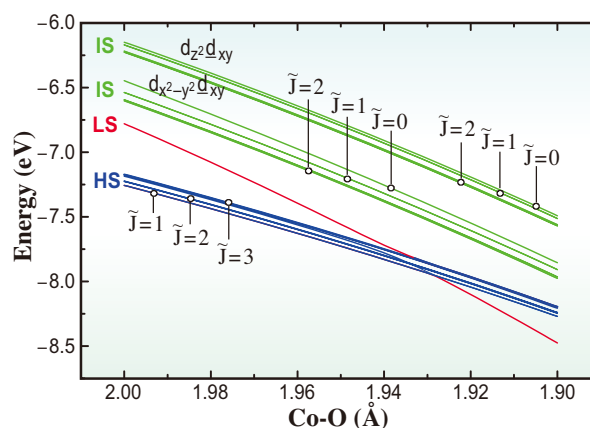


Fig. 3. Energy level diagram of a CoO_6 cluster as a function of the Co-O distance.

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References

- [1] J.B. Goodenough and P.M. Raccach: J. Appl. Phys. **36** (1965) 1031.
- [2] M.W. Haverkort *et al.*: Phys. Rev. Lett. **97** (2006) 176405
- [3] J.-M. Chen, Y.-Y. Chin, M. Valldor, Z. Hu, J.-M. Lee, S.-C. Haw, N. Hiraoka, H. Ishii, C.-W. Pao, K.-D. Tsuei, J.-F. Lee, H.-J. Lin, L.-Y. Jang, A. Tanaka, C.-T. Chen and L. H. Tjeng: J. Am. Chem. Soc. **136** (2014) 1514.
- [4] J.P. Rueff *et al.*: Phys. Rev. B **73** (2006) 024424.

Fracture-induced amorphization of polycrystalline stishovite: Amorphization makes the ceramic tough

Silicon dioxide (SiO_2) has eight stable crystalline phases at conditions of the Earth's rocky parts. Many metastable phases including amorphous phases have been known, which indicates the presence of large kinetic barriers. As a consequence, some crystalline silica phases transform to amorphous phases by bypassing the liquid via two different pathways: heating under atmospheric pressure (temperature-induced amorphization) and compression at room temperature (pressure-induced amorphization). In the present study, we discovered a new pathway, a fracture-induced amorphization of stishovite that is a high-pressure polymorph [1]. The amorphization accompanies a huge volume expansion of $\sim 100\%$ and occurs in a thin layer whose thickness from the fracture surface is several tens of nanometers. Amorphous silica materials that look like strings or worms were observed on the fracture surfaces. The amount of amorphous silica near the fracture surfaces is positively correlated with fracture toughness (K_{IC}): in other words, the nanometer scale amorphization makes this material tough. A similar toughening mechanism has been known for a popular ceramic material, zirconia. In the case of zirconia, a huge tensile stress to cause fracture induces tetragonal to monoclinic transition. This transition accompanies a volume expansion of $\sim 4\%$. This volume expansion generates compressional stress against the crack opening, which makes the material tougher (transformation toughening) [2]. In the case of stishovite, the huge volume expansion associated with fracture-induced amorphization is the origin of the toughening.

Stishovite is a high-pressure polymorph in silicon dioxide and is stable between 9 and 70 GPa. In 1996, it was reported that stishovite is the hardest oxide that can be utilized at ambient conditions [3]. The reported hardness values were ~ 30 GPa, which is 1.5 times as large as that of alumina, which is known as a hard ceramic and widely used in industry. However, stishovite has not been used for industrial applications because single-crystal stishovite is a very brittle material ($K_{\text{IC}} = 1.6 \text{ MPa}\cdot\text{m}^{1/2}$). In general, hardness and toughness are mutually exclusive: hard materials are generally brittle (easy to break). The brittleness of single crystal stishovite hinders the usage in industry.

In 2012, we reported the synthesis of nanocrystalline bulk stishovite with very high fracture toughness ($K_{\text{IC}} > 10 \text{ MPa}\cdot\text{m}^{1/2}$) [4] (Fig. 1). Since single-crystal stishovite is very brittle, a toughening mechanism should be activated in the nanocrystalline bulk stishovite materials. However, the question “what does make this material tough?” had

remained unanswered. In the previous paper [4], we reported that fracture surfaces of this material look very different from those of other ceramics such as alumina. The fracture surfaces of this material show “worm-like texture.” I thought that this unusual fracture surface can be an important key to answer this question.

In order to examine phenomena that happened on the fracture surfaces of nanocrystalline bulk stishovite, we performed Si-K XANES measurements for a series of stishovite polycrystals at soft X-ray photochemistry beamline BL27SU (Fig. 2). For each measurement, we collected two spectra simultaneously: a spectrum collected by the total electron yield (TEY) method and that by the partial fluorescence yield (PFY) method. The former is a surface-sensitive method: a XANES spectrum to a depth of ~ 10 nm from the surface can be obtained. The latter is a bulk-sensitive method: a spectrum to a depth of $\sim 1 \mu\text{m}$ can be obtained for silica materials. Since the peak position of XANES spectra of stishovite with silicon coordination number (CN) of 6 is quite different from those of silica materials with CN=4, these spectra can be used as fingerprints to distinguish silica materials with different CNs.

First, we performed a measurement for an unpolished (as sintered) surface of a nanocrystalline bulk stishovite material (v). The TEY and PFY spectra can be explained by the presence of a single phase of stishovite. On the other hand, two peaks indicating the presence of materials with CN=4 and 6 were clearly observed in a TEY spectrum obtained from a polished surface of a stishovite polycrystal (vi). Note that the CN=4 peak cannot be observed in the PFY spectrum. These results demonstrate that the CN=4 materials coexist with stishovite (CN=6) in a thin layer whose thickness is several tens of nanometers from the polished surface. A previous study reported that stishovite was inverted

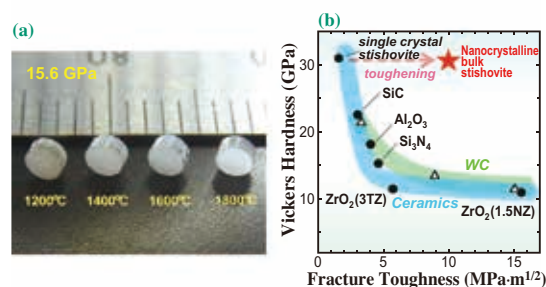


Fig. 1. (a) A photograph of some representative samples of nanocrystalline bulk stishovite. (b) Relationship between hardness and fracture toughness of hard materials, tungsten carbides and ceramics. Single-crystal stishovite is brittle and nanocrystalline bulk stishovite has enhanced fracture toughness. A toughening mechanism is activated for nanocrystalline bulk stishovite.

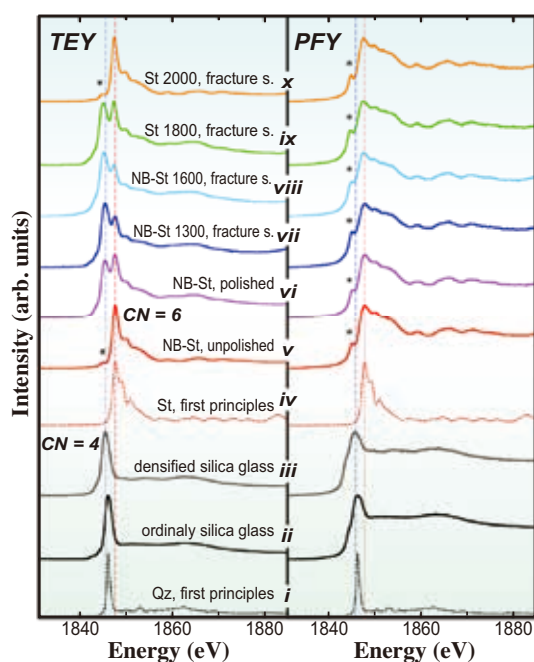


Fig. 2. Representative Si-K XANES spectra of a series of stishovite polycrystals and those obtained by first-principles calculations. TEY and PFY spectra collected simultaneously from one measurement are shown side by side. (i), a-quartz by first-principles calculation; (ii), ordinary silica glass; (iii) densified silica glass; (iv), stishovite by first-principles calculation; (v), unpolished surface of a polycrystalline stishovite sample with synthesis temperature (T_s) of 1300°C; (vi), polished surface $T_s = 1300^\circ\text{C}$; (vii), fracture surface $T_s = 1300^\circ\text{C}$; (viii), fracture surface $T_s = 1600^\circ\text{C}$; (ix), fracture surface $T_s = 1800^\circ\text{C}$; (x) fracture surface $T_s = 2000^\circ\text{C}$. *, a pre-edge peak of stishovite.

to silica glass (CN=4) by mechanical grinding, which supports our observations. Similar features were observed in the spectra obtained from fracture surfaces of nanopolycrystalline bulk stishovite materials synthesized at different temperatures (vii, viii), demonstrating the coexistence of the CN=4 material with stishovite (CN=6) in thin layers (several tens of nanometer thick) adjacent to the fracture surfaces. We observed a decrease of relative intensity of the CN=4 peak with respect to the CN=6 peak intensity in TEY spectra with increasing synthesis temperature (T_s) above 1700°C (ix). At the highest T_s of 2000°C, we observed the complete absence of the CN=4 peak (x). The fracture surface of this sample did not show “worm-like texture.” The fracture surface looks similar to that of alumina showing intergranular fracture. The results of XANES measurements clearly show that thin layers (several tens of nanometer thick) which contain CN=4 silica material exist on fracture surfaces of nanocrystalline bulk stishovite.

In order to identify the CN=4 phase, we performed TEM and SEM observations (Fig. 3) of crashed pieces of nanocrystalline bulk stishovite. A low magnification image shows the presence of worm-like textures (Fig. 3(a)). Selected-area electron diffraction patterns of the worm-like textures have a halo and an example of

X-ray fluorescence spectra show the presence of only silicon and oxygen (Fig. 3(b)). These results indicate that fracture of nanocrystalline bulk stishovite induces transformation from stishovite (CN=6) to amorphous silica (CN=4), which results in the coexistence of the CN=4 and CN=6 peaks in the TEY-XANES spectrum. Figure 3(c) shows a high-magnification SEM image of the amorphous silica that exists on the fracture surface of the nanocrystalline bulk stishovite.

Nanocrystalline bulk stishovite with simultaneous high-hardness and high-toughness with composition of SiO_2 meets the requirement for managing the scarcity of chemical elements [5].

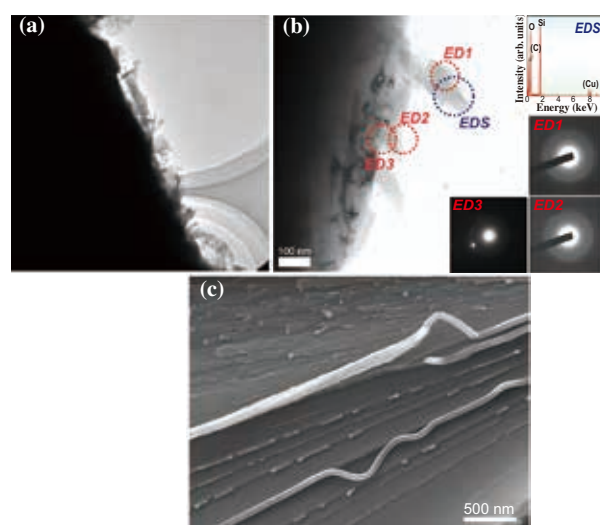


Fig. 3. Amorphous silica on fracture surfaces of nanocrystalline bulk stishovite. (a) A low-magnification TEM micrograph of worm-like texture on a fracture surface of a broken piece of nanocrystalline bulk stishovite. (b) A magnified TEM image of worm-like textures. Electron diffraction patterns (ED1 and 2) and an X-ray fluorescence spectrum by energy dispersive X-ray spectroscopy (EDS) show presence of an amorphous silica phase on the fracture surface. An electron diffraction pattern (ED3) that is collected at the interface between the amorphous and stishovite crystal shows coexistence of halo and diffracted spots from a stishovite grain. (c) A magnified SEM micrograph on a fractured stishovite grain. String-shaped amorphous silica materials are observed. They could be one of the sources of the worm-like texture.

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References

- [1] N. Nishiyama, F. Wakai, H. Ohfuji, Y. Tamenori, H. Murata, T. Taniguchi, M. Matsushita, M. Takahashi, E. Kulik, K. Yoshida, K. Wada, J. Bednarcik, T. Irifune: *Sci. Rep.* **4** (2014) 6558.
- [2] R.C. Garvie *et al.*: *Nature* **258** (1975) 703.
- [3] J.M. Léger *et al.*: *Nature* **383** (1996) 401.
- [4] N. Nishiyama *et al.*: *Scripta Mater.* **67** (2012) 955.
- [5] E. Nakamura and K. Sato: *Nat. Mater.* **10** (2011) 158.

Complementary use of soft X-ray operando spectromicroscopies on the developments of next-generation devices

The developments of next-generation devices, such as 2D electron systems that includes AlGaIn/GaN and graphene, is becoming increasingly important to supplement device functions of Si-based electronics, which have already reached the 15-nm design rules. Graphene has been extensively studied as a next-generation device material owing to its suppression of short-channel effects, which are desirable to reduce device size and its excellent electronic properties, such as giant carrier mobilities and the absence of diminished backscattering.

We have conducted research on graphene from wafer-scale growth [1] to transistor fabrication [2], and obtained a world-record high-frequency characteristic for graphene transistors (GFET) [2]. It is, however, inferred that parasitic resistances, such as the contact or access resistance, considerably degrade device performance [2]. The interfaces related with the parasitic resistances are responsible for the degradation. Furthermore, the impact of the interfaces on the device performances varies with the operation conditions, e.g., gate bias (Fermi level (E_F)), which makes GFET designation difficult. Because the development of the next-generation devices requires microscopic information on the electronic states near the interfaces under operational conditions, we have adopted operando spectromicroscopy to bridge the gap between the material and the device (Fig. 1).

Here we demonstrate how the complementary use of soft X-ray spectromicroscopies, 3D scanning photoelectron microscopy (3D nano-ESCA) at BL07LSU [3], and photoemission electron microscopy (PEEM) at BL17SU [4] is powerful tool to probe the electronic states, i.e., both conduction and valence bands of GFET, under operational conditions. One of our main targets is the interface between graphene and SiO_2 because channel-gate oxide interfaces are at the heart of field-effect. In addition, the interface between graphene and a metal contact is crucial because the contact resistance contributes non-negligibly to a low channel resistance.

To examine the validity of operando 3D nano-ESCA for the interface with gate oxide, the shift of E_F at the center of the graphene channel by the gate bias application was examined in detail by pinpoint C 1s core level photoelectron spectroscopy (Fig. 2(a)) [3].

Qualitatively, the C 1s peak of graphene is red-shifted by a negative gate bias application, which lowers E_F . For further quantitative analysis, the data were reproduced by a theoretical curve based on a simple capacitance model and linear DOS (Fig. 2(b)). One of the extracted parameters, the gate bias at the charge neutrality point, V_{CNP} , agrees with that extracted from the macroscopic drain current — gate bias curve. The value of another extracted parameter, $E_{\text{BE}}(\text{DP})$, which is the binding energy of an electrically neutral graphene channel, is consistent with the binding energy of electrically-neutral graphite. Thus, the field-effect of GFET is probed directly by operando 3D nano-ESCA.

The change in the conduction bands of GFET under a gate-bias application was investigated with microscopic X-ray absorption spectroscopy (μ -XAS) by collecting Auger-electron images near the C 1s absorption edge [4]. The μ -XAS spectra at the center of the GFET channel is changed by the gate-bias application (Fig. 2(c)). The π^* peak change indicates that the DOS in the conduction band is altered. This observation cannot be explained within the framework of the so-called initial-state approximation. Instead, the final-state is responsible for the spectral change. More specifically, many-body effects due to core holes, i.e., the excitonic effect and Anderson orthogonality catastrophe, modify the π^* peak lineshape. The degree of the many-body effect depends on the density of states near E_F . Thus, operando PEEM through μ -XAS

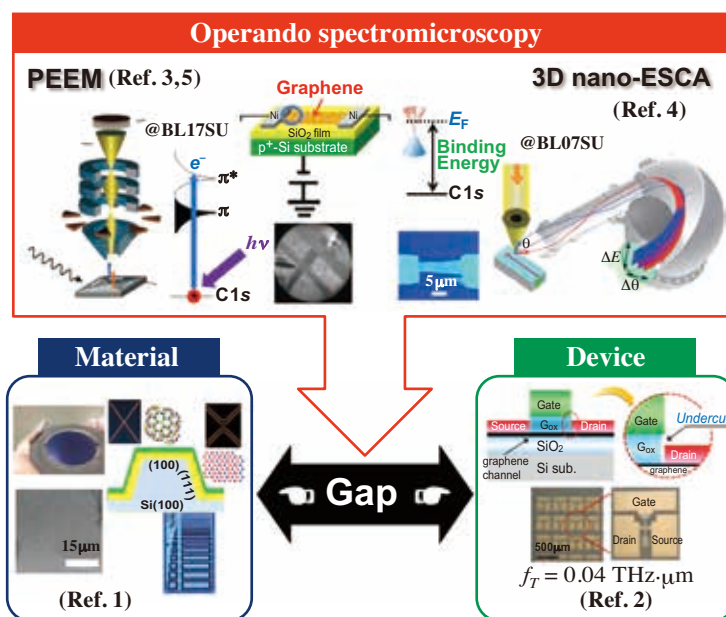


Fig. 1. Role of operando spectromicroscopy.

can probe microscopic information in the conduction bands and E_F .

The presence of a charge transfer region (CTR) near the metal contact is a serious issue in developing high-performance GFET. The CTR is speculated to be wide ($< 1 \mu\text{m}$) due to the vanishing DOS near the Dirac point, which makes analysis of the contact resistance difficult [5]. The CTR was investigated by 3D nano-ESCA (Fig. 3(a)) [5]. The binding energy of graphene is reduced near the metal contact. This indicates that the electron transfer from graphene to the contact metal spans a wide region ($\sim 1 \mu\text{m}$) and provides evidence for the presence of the CTR.

The CTR was investigated in more detail by operando PEEM (Fig. 3(b)) [4]. Consistent with 3D nano-ESCA, the existence of the CTR is inferred from the π^* peak change which reflects the E_F change. More interestingly, it is clarified that the gate bias application narrows the width of the CTR. This can be explained by considering the fact that additional carrier doping by the gate bias reduces the screening length in the CTR.

In summary, the complementary use of soft X-ray operando spectromicroscopies reveals local variations in the electronic states near device interfaces, which cannot be elucidated from device simulations and consequently be considered in device design. The significance of the operando spectromicroscopy is now

recognized, and has resulted in its adoption as a target in the NEDO academic-industrial alliance project with Sumitomo Electric Industries.

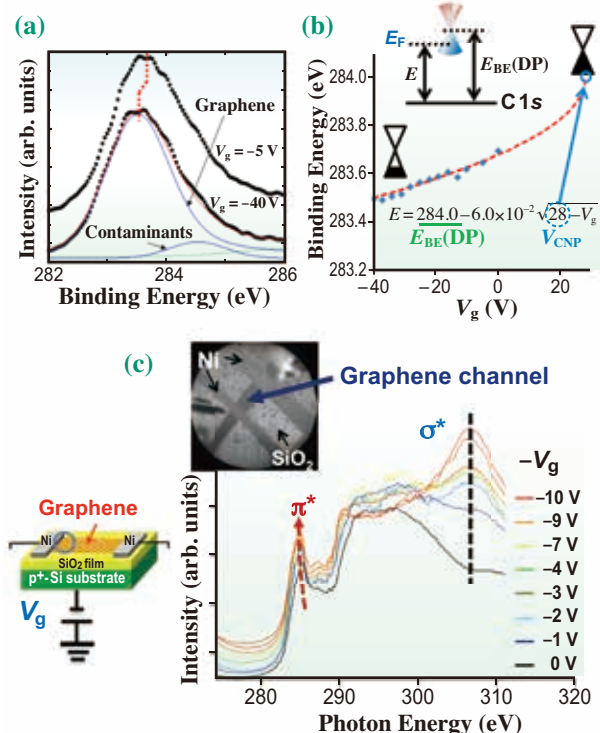


Fig. 2. Operando observations of field-effect by (a, b) 3D nano-ESCA [3] and (c) PEEM [4]. Blue rhombi and red dotted line in (b) are experimental data and the fitted curve, respectively.

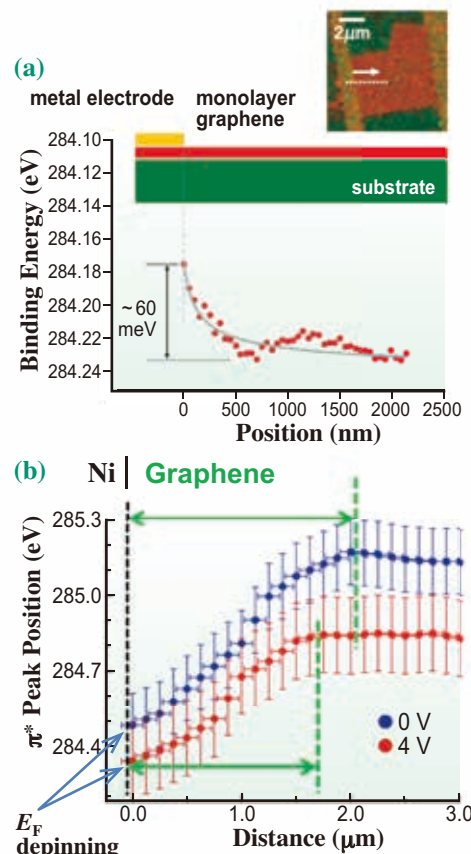


Fig. 3. Observation of metal contact by (a) 3D nano-ESCA [5] and (b) operando PEEM [4].

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References

- [1] H. Fukidome *et al.*: Proc. IEEE **101** (2013) 1557.
- [2] M.H. Jung *et al.*: Proc. IEEE **101** (2013) 1603.
- [3] H. Fukidome, K. Nagashio, N. Nagamura, K. Tashima, K. Funakubo, K. Horiba, M. Suemitsu, A. Toriumi and M. Oshima: Appl. Phys. Exp. **7** (2014) 065101.
- [4] H. Fukidome, T. Ide, Y. Kawai, T. Shinohara, N. Nagamura, K. Horiba, M. Kotsugi, T. Ohkouchi, T. Kinoshita, H. Kumigashira, M. Oshima and M. Suemitsu: Sci. Rep. **4** (2014) 3713.
- [5] N. Nagamura *et al.*: Appl. Phys. Lett. **102** (2013) 246104.

How important is the surface potential barrier to determine the lifetime of photoexcited carriers on TiO₂ photocatalysts?: Time-resolved photoelectron spectroscopy study

Since Fujishima and Honda discovered the electrochemical photolysis of water at the titanium dioxide (TiO₂) electrode in 1969 [1], extensive efforts have been devoted to elucidate physics behind the superior photocatalytic properties of TiO₂. Photocatalytic reactions are initiated by the interaction of photoexcited carriers (electrons and holes) with chemical species on the catalyst surface (Fig. 1). Thus, the efficiency of photocatalysis should depend on the carrier lifetime, which is determined by a balance between electron-hole separation and electron-hole recombination processes.

TiO₂ crystals with anatase and rutile structures are usually used as photocatalysts. The different carrier lifetimes of the polymorphs suggest that anatase is more photocatalytically active than rutile [2]. However, the experimentally determined lifetimes range from picoseconds to microseconds. Although the measurement conditions, such as temperatures, surrounding environment, crystal size, etc., are known to affect the lifetime, there is no reasonable explanation for the diversity of the reported values. What governs the carrier lifetime is an important question that must be answered in order to comprehensively understand the photocatalytic activity of TiO₂.

A major difficulty in carrier dynamics studies is assessing the effect of the space charge layer (SCL) on the carrier behavior. In the SCL, the band positions vary energetically when going from the bulk to the surface, and this energy difference is viewed as a potential barrier for charged particles. Photoexcited carriers must overcome the surface potential barrier as they are transported to the surface to initiate chemical

reactions (Fig. 1). Thus, the surface potential should significantly influence the photocatalytic activity via the carrier lifetime. However, most studies do not take the role of the SCL into account when discussing the activity from the carrier dynamics point of view [2].

To clarify the role of the surface potential in the SCL, time-resolved (TR) photoelectron spectroscopy (PES) was utilized to measure the carrier lifetime on single-crystalline anatase and rutile surfaces [3]. Compared to other transient measurement techniques, e.g., photoluminescence spectroscopy, absorption spectroscopy, and photoconductivity measurements, this technique is advantageous because the carrier lifetime and potential barrier height can be determined, allowing correlations between these two factors to be estimated. The TR-PES measurements were carried out at BL07LSU utilizing the laser-pump/synchrotron-radiation-(SR)-probe method [4]. A second harmonic of an amplified Ti:sapphire laser (the photon energy of 3.06 eV with a pulse width 35 fs) was used to generate excited carriers, and 600-eV SR pulses were employed to measure the PES spectra. The laser pulse excites the valence electrons to the conduction band, generating the electron-hole pairs (Fig. 2(a)). The electrons and holes are spatially separated by the electric field within the SCL; one drifts to the surface and is trapped by the surface-trap states, and the other is transported to the bulk (Fig. 2(b)). The charge separation induces a surface photovoltage (SPV), whose magnitude diminishes over time as the density of the surface-trapped carriers decreases due to the recombination process. The counterpart carriers are thermally diffused to the surface by overpassing the potential barrier (Fig. 2(c)). We examined the time-dependent change of the SPV by measuring the temporal variations of the energy shift in the PES spectra.

Figure 3(a) shows the energy shifts of the Ti 2p_{3/2} core-level peaks of the anatase TiO₂(001) and rutile TiO₂(110) surfaces. Large shifts of 0.1–0.2 eV are induced after a 0.2 ns delay time due to the time interval between the pump and probe pulses but the magnitude of the shifts decreases gradually over the time range of about 100 ns. The estimated relaxation times (τ_s) of the SPV are 50 ± 30 ns for anatase and 180 ± 100 ns for rutile. Here, τ_s is defined as the relaxation time of the SPV in the dark condition; namely, it is the hypothetical lifetime of the trapped carriers on the crystal surface with a potential barrier height (V_s) in the absence of the SPV [3]. On the anatase and rutile surfaces, the charge

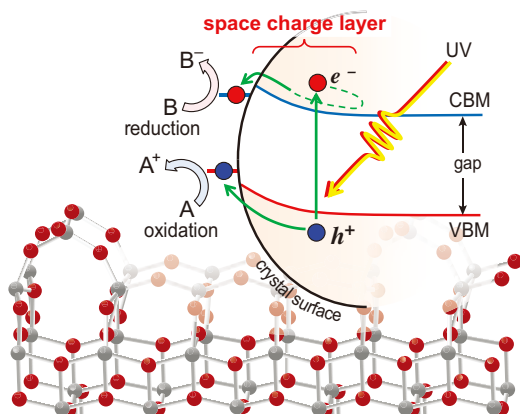


Fig. 1. Schematic of the carrier dynamics on a photocatalyst surface from photoexcitation to redox reactions.

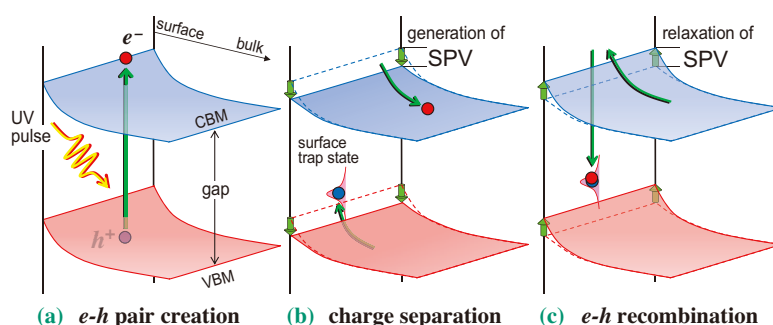


Fig. 2. Energy band diagram of the surface photovoltage (SPV) effect. (a) Photoexcitation of the electron-hole ($e-h$) pair; (b) charge separation and the generation of the SPV, and (c) SPV relaxation by charge recombination.

accumulation layers are formed with V_s values of 0.2 and 0.4 eV, respectively. The larger τ_s for rutile is surely a consequence of the larger V_s due to the smaller probability of the counterpart carriers overcoming the potential barrier. If we compare the carrier lifetimes on both surfaces with the same V_s , the carrier lifetime on the anatase surface is always larger than that on the rutile surface (Fig. 3(b)). The surface-trapped carriers with longer lifetimes are more likely to encounter adsorbed species and initiate chemical reactions. This is in accordance with the well-known tendency that

anatase TiO_2 is photocatalytically more active than rutile TiO_2 .

Another important implication in Fig. 3(b) is that the carrier lifetime varies from picoseconds to microseconds depending on V_s . Thus, strategy to enhance the photocatalytic activity is to enlarge V_s , for example, by chemically modifying the crystal surface. Such a potential-barrier engineering is also applicable for photovoltaics, where the charge separation efficiency and the carrier lifetime are determining factors for photoelectric conversion efficiency.

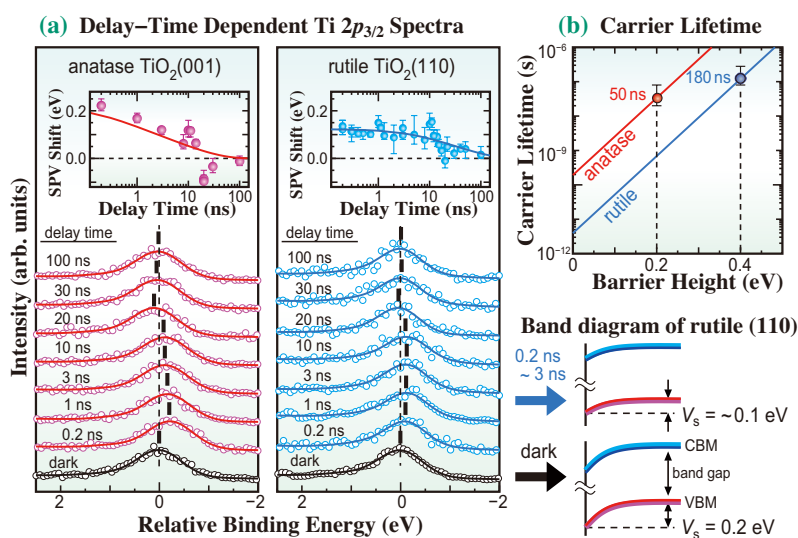


Fig. 3. (a) Change in the Ti $2p_{3/2}$ spectra of anatase $\text{TiO}_2(001)$ and rutile $\text{TiO}_2(110)$ as a function of the delay time. Pump and probe lights are a 3.06-eV laser with a power density of 12 mJ/cm²/pulse and a 600-eV SR light, respectively. Insets show the relaxation of the SPV shift. Energy band diagrams of the rutile surface in a dark condition. Lower right shows those at the delay times between 0.2 and 3 ns. (b) Potential-barrier-height dependence of the carrier lifetimes on the anatase (001) and rutile (110) surfaces.

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References

- [1] A. Fujishima *et al.*: Kogyo Kagaku Zasshi **72** (1969) 108. (in Japanese)
- [2] M. Xu *et al.*: Phys. Rev. Lett. **106** (2011) 138302.
- [3] K. Ozawa, M. Emori, S. Yamamoto, R. Yukawa, Sh. Yamamoto, R. Hobara, K. Fujikawa, H. Sakama, I. Matsuda: J. Chem. Phys. Lett. **5** (2014) 1953.
- [4] S. Yamamoto *et al.*: J. Phys. Soc. Jpn. **82** (2013) 021003.

Novel transition from dimer Mott to charge order phase in a quarter-filled organic salt

Internal degrees of freedom are a central idea penetrating into diverse fields of fundamental physics. In condensed matter physics, an electron in solids possesses the charge, spin and orbital degrees of freedom, each of which exhibits various ordered states at low temperatures. A quintessential example is an ordered state of the spin degree of freedom manifesting itself in a permanent magnet. A unique feature in this field is that a novel internal degree of freedom can be artificially introduced in some complex solids. In a crystal composed of clusters, the intra-cluster lattice vibration called “rattling” is observed and controlled.

The cluster degree of freedom closely couples with the electron correlation effect, leading to intriguing electronic states. This is seen in a family of the quasi two-dimensional organic salt R_2X (R_2 : a dimer organic molecule, X : an anion), where one hole from the monovalent anion X^- exists per dimer (per two donor molecules). Basically, such a quarter-filled nature leads to a metallic state (Fig. 1(a)), but the strong correlation effect varies the system drastically. As shown in Fig. 1(b), in the weakly dimerized case, the correlated hole is localized on the molecular site owing to the long-range nature of Coulomb interaction to form a charge order. On the other hand, under strong dimerization, the hole is localized on the dimer to act as a Mott insulator (dimer Mott insulator, Fig. 1(c)). Theoretically, these two insulating states are suggested to change

into each other by tuning materials parameters such as the transfer energy and the Coulomb repulsion [1]. Thus, the materials located near the border of such insulating phases may have an instability toward another insulating state. This instability originates from the charge degrees of freedom in the dimer, which can be regarded as a cluster structure in the crystal. Recent dielectric measurements indeed suggested the instability to a charge order hidden in a κ -type dimer Mott insulator [2]. However, there is no direct spectroscopic evidence of charge disproportionation, leaving it controversial whether the instability toward the charge order exists in the dimer Mott phase. To examine this issue, we here explore the opposite case where the instability to the dimer Mott phase is hidden in a charge-ordered dimer-type organic salt through the infrared microspectroscopy performed at beamline BL43IR [3].

We focus on the dimer-type organic salt β -(*meso*-DMBEDT-TTF)₂PF₆ (R =*meso*-DMBEDT-TTF, X =PF₆), which exhibits a charge order below $T_c = 70$ K. The charge order pattern determined by the synchrotron radiation X-ray study is of checkerboard type (left inset of Fig. 2). We note that the checkerboard-type charge order can be regarded as an electric dipole order, because the charge disproportionation occurs within a dimer. The main panel of Fig. 2 displays the optical conductivity spectra obtained from the Kramers-Kronig analysis for the reflectivity data. The distinct feature newly found is a pronounced peak structure at around 0.6 eV, which is enhanced with lowering temperature from 300 K down to T_c . This mid-infrared peak can be assigned to a dimer peak, a transition from bonding to anti-bonding orbitals of dimerized molecules, as widely observed in dimerized organic salts. Importantly, the dimer-peak intensity is enhanced with decreasing temperature in the dimer Mott insulating phase, while it is reduced in a correlated metallic phase [4]. The enhancement of the dimer-peak intensity down to T_c then indicates that the high-temperature phase in this material can be regarded as a dimer Mott insulating phase (right inset of Fig. 2). Thus, the charge order transition at T_c is a transition from dimer Mott to charge order phase due to the internal change degree of freedom in the dimer.

An intriguing question is how these two correlated insulating phases compete in real space. To address this, we show the positional dependence of the local reflectivity spectra measured with high spatial resolution of 10 μm using high-brilliance synchrotron

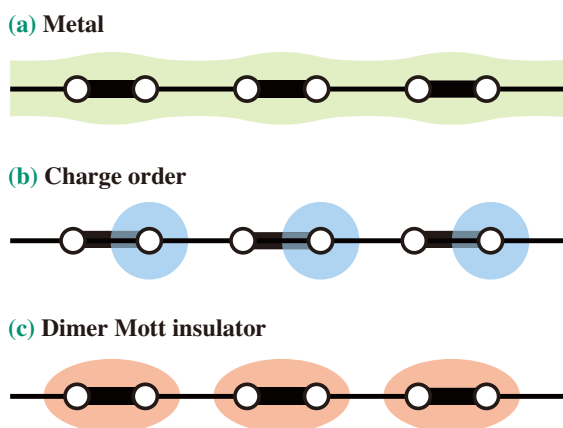


Fig. 1. Schematic electronic states in the quarter-filled organic salt R_2X . The open circles represent the organic molecules R and thick line shows the dimerization of two molecules. The color-shaded area represents the electron distribution. The closed-shell anion X is omitted. (a) A metallic state in weak correlation case. (b) The charge ordered state induced by the long-range Coulomb repulsion. (c) The dimer Mott insulating state.

radiation light. We here evaluate the local reflectivity ratio $R(184.5 \text{ meV})/R(181 \text{ meV})$ at each point, which gives a relative strength between the charge ordered insulating state with 184.5-meV peak and the dimer Mott insulating state with 181-meV peak [5], and plot its spatial distribution in Fig. 3. The measured surface area is $1400 \times 100 \mu\text{m}^2$ and the red- and blue-color areas indicate the dimer Mott and the charge order states, respectively. In contrast to spatially-homogeneous spectra above T_c , the low-temperature spectra are highly inhomogeneous, consisting of the competitive dimer Mott and charge order insulating states.

Obviously, the present phenomenon is different from conventional phase transition occurring at finite temperatures. The spatial coexistence consisting of dimer Mott and charge order states appears even at 10 K far below T_c , indicating that the phase transition in this material is not driven by entropy term in the free energy. We rather suggest that the materials parameters are largely varied with temperature to drive the transition. This type of transition may possess quantum nature in the sense that the transition is driven by the materials parameters. As theoretically suggested, the ground state of the two-dimensional quarter-filled organic system varies from dimer Mott to charge order state by tuning such parameters [1]. The observed spatial inhomogeneity therefore implies that this organic salt actually lies on the border between those two correlated insulating ground states in the parameter space.

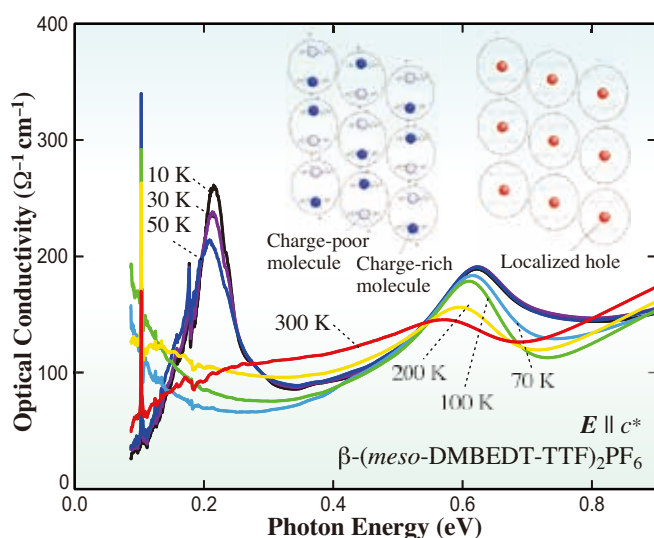


Fig. 2. The optical conductivity spectra measured at various temperatures with polarization parallel to the c^* axis. The left and right insets illustrate the charge order ($T < T_c = 70 \text{ K}$) and the dimer Mott insulator ($T > T_c$) emerged in the two-dimensional molecular plane of β -(*meso*-DMBEDT-TTF) $_2$ PF $_6$, respectively. Two donor molecules circled by the dotted ellipsoid are dimerized.

The anomalous transition phenomenon shown in the present study originates from the internal cluster degree of freedom, which is introduced by a unique dimer structure in the organic systems. Such a characteristic structure is also found in transition-metal oxides such as BaIrO $_3$ and Ba $_4$ Ru $_3$ O $_{10}$, which are consisting of Ir $_3$ O $_{12}$ and Ru $_3$ O $_{12}$ trimers, respectively. Interestingly, both compounds exhibit unusual phase transitions, in which the underlying cluster degree of freedom may play a crucial role as a ubiquitous property in the complex solids.

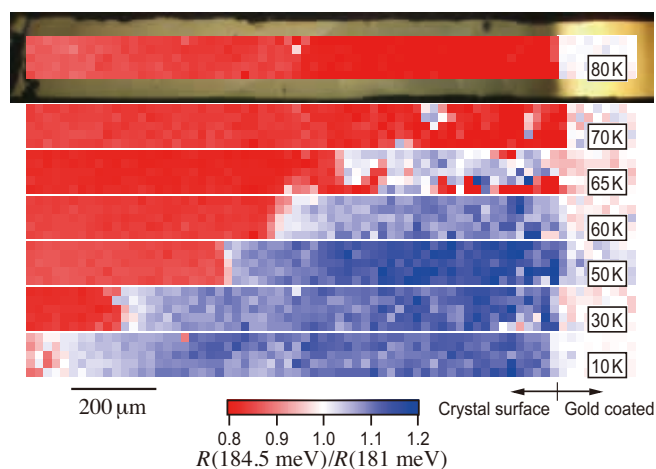


Fig. 3. The reflectivity-ratio mapping on the crystal surface of $1400 \times 100 \mu\text{m}^2$ measured at several temperatures. The color scale shows the reflectivity ratio $R(184.5 \text{ meV})/R(181 \text{ meV})$ at each position. Red and blue areas indicate the dimer Mott and the charge ordered states, respectively. The right-side white region is a gold-coated area for measuring reference spectrum.

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References

- [1] H. Seo: J. Phys. Soc. Jpn. **69** (2000) 805.
- [2] M. Abdel-Jawad *et al.*: Phys. Rev. B **82** (2010) 125119.
- [3] R. Okazaki, Y. Ikemoto, T. Moriwaki, T. Shikama, K. Takahashi, H. Mori, H. Nakaya, T. Sasaki, Y. Yasui and I. Terasaki: Phys. Rev. Lett. **111** (2013) 217801.
- [4] T. Sasaki *et al.*: Phys. Rev. B **69** (2004) 064508.
- [5] M. Tanaka *et al.*: J. Phys. Soc. Jpn. **77** (2008) 024714.

Synchrotron radiation-based Mössbauer spectra of ^{174}Yb measured with internal conversion electrons

Mössbauer spectroscopy is a well-established method in physics, chemistry, biology, and earth science, since it provides element-specific information on the electronic states, such as valence and magnetism in complex materials. Most Mössbauer studies are performed using ^{57}Fe and ^{119}Sn with radioactive isotopes (RI) sources, although the Mössbauer effect has been observed in approximately 100 nuclides of nearly 50 elements (See Fig. 1). One major difficulty in Mössbauer spectroscopy is preparation of RI as γ -ray sources, but this can be avoided using synchrotron radiation (SR); we can select X-rays with the appropriate energy for the Mössbauer effect. Moreover, SR has high brilliance and thus the electronic states can be studied under extreme conditions like high pressure. SR-based Mössbauer spectroscopy developed in 2009 is one such method in the energy domain [1], and measurements using many nuclides are promising (Fig. 1). In fact, ^{151}Eu Mössbauer study has already been performed under high pressure with this method [2]. However, the measurements are often time consuming. To reduce the measurement time, we aimed to enhance the counting rate of the emission from the nuclear excited state. From the nucleus resonantly excited, γ -rays or internal conversion (IC) electrons are emitted at the de-excitation. In previous measurements, the IC electrons were not detected, while the γ -rays or the fluorescent X-rays that accompany the IC electron emission were detected, because the X-ray windows of the cryostat and the detector shield the electrons. Therefore, we developed a detection system using an X-ray-windowless avalanche photodiode (APD) detector and a sample chamber with a He-flow cryostat [3]. The developed system is applicable to SR-based Mössbauer spectroscopy for the 76.5 keV excited state of ^{174}Yb . ^{174}Yb is the most abundant Yb nuclide (natural abundance of 31.4%), but is not the most popular for Yb Mössbauer spectroscopy due to difficulties in preparing its RI source [4]. If ^{174}Yb

Mössbauer spectroscopy is possible, then expensive isotope enrichment is unnecessary.

The experimental setup for SR-based Mössbauer spectroscopy is shown in Fig. 2. The experiments were performed at beamlines BL09XU and BL11XU. The electron-storage ring's operating mode was the "203 bunch" mode. SR with the energy of the ^{174}Yb nuclear resonance was transmitted by a sample in the cryostat (the "transmitter" in Fig. 2). Then the SR was scattered by a sample in another cryostat (the "scatterer" in Fig. 2). The scatterer was moved using a velocity transducer to measure the Mössbauer spectrum at low temperatures. In the cryostat, an eight-element APD detector was combined to its flange on one side (Fig. 2 upper right) and arranged just above the scatterer under vacuum, so that electrons can reach the detector. Thus, both the emitted IC electrons and X-rays from the scatterer can be detected with the APD. Because nuclear resonant scattering (NRS) by ^{174}Yb is emitted with a delay (half-life $t_{1/2}=1.79$ ns), NRS was counted in the time window between 6.1 ns and 13.6 ns after the SR incidence. In this system, either the transmitter or the scatterer is studied, while the other is used as the energy standard. In this study, both the transmitter and scatterer were natural YbB_{12} powdered from a single crystal [5] because the large recoilless fraction of YbB_{12} is advantageous when observing a clear signal in the Mössbauer spectrum. The transmitter thickness was $60.9 \text{ mg/cm}^2 \text{ YbB}_{12}$, while that of the scatterer was $736 \text{ mg/cm}^2 \text{ YbB}_{12}$. The transmitter was set to 20 K and the scatterer was set to 26 K in a $<0.01 \text{ Pa}$ vacuum.

The ^{174}Yb Mössbauer spectrum observed within 10 hours is shown in Fig. 3. The NRS counting rate was 6 cps, while it was 1.2 cps in our previous ^{174}Yb SR-based Mössbauer spectroscopy using an eight-element APD with an X-ray window. Therefore, the counting rate was five times higher than that of the previous system. The spectrum was evaluated using the formulae in Ref. 1. Here, absorption spectrum

H																	He
Li	Be	Elements including Mössbauer active nuclide										B	C	N	O	F	Ne
Na	Mg	Elements without Mössbauer active nuclide										Al	Si	P	S	Cl	Ar
K	Ca	Sc	Ti	V	Cr	Mn	Fe	Co	Ni	Cu	Zn	Ga	Ge	As	Se	Br	Kr
Rb	Sr	Y	Zr	Nb	Mo	Tc	Ru	Rh	Pd	Ag	Cd	In	Sn	Sb	Te	I	Xe
Cs	Ba	*	Hf	Ta	W	Re	Os	Ir	Pt	Au	Hg	Tl	Pb	Bi	Po	At	Rn
Fr	Ra	**	104~														
*Lanthanide		La	Ce	Pr	Nd	Pm	Sm	Eu	Gd	Tb	Dy	Ho	Er	Tm	Yb	Lu	
**Actinide		Ac	Th	Pa	U	Np	Pu	Am	Cm		Cf	Es	Fm	Md	No	Lr	

Fig. 1. Table of elements including Mössbauer active nuclides.

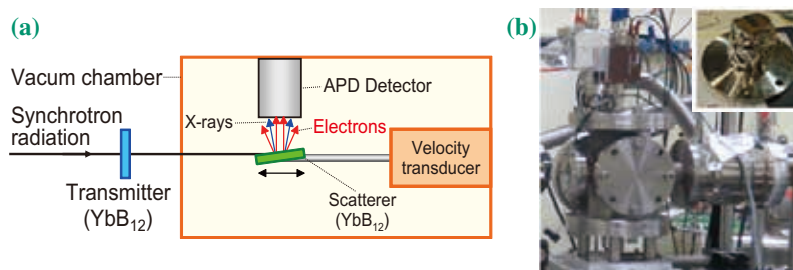


Fig. 2. (a) Schematic drawing of the developed X-ray windowless system for the SR-based Mössbauer spectroscopy, (b) photo of the vacuum chamber, and (inset in b) the X-ray windowless APD detector.

narrows owing to the time window, which leads to an improved spectrum resolution. The full width at half maximum (FWHM) of this spectrum is 1.3 ± 0.2 mm/s. Because the narrowest ideal width in conventional RI Mössbauer spectroscopy is 2.0 mm/s at FWHM for the nuclear level of ^{174}Yb , the FWHM of the observed spectrum is narrower than the conventional ideal width. In some cases, this narrowing effect is useful to experimentally determine the energy shifts in Mössbauer spectra; the difference in isomer shifts between Yb^{2+} and Yb^{3+} is less than 0.2 mm/s in the ^{174}Yb spectrum, and consequently, the line width is critical.

The detection system developed here can also be applied to the SR-based Mössbauer spectroscopy using other nuclides. In fact, many nuclides in Fig. 1 also have high IC coefficients, and this system should realize drastic improvement of the counting rates in Mössbauer spectroscopy with these nuclides. Especially, rare-earth elements except Ce have Mössbauer active nuclides

with high IC coefficients. Therefore, the SR-based Mössbauer spectroscopy using this system should be utilized to study the electronic states (especially $4f$) of the rare-earth compounds.

In summary, the IC electron detection system developed here dramatically enhances the counting rate in SR-based Mössbauer spectroscopy enabling efficient measurements. ^{174}Yb Mössbauer spectroscopy was performed using this system, and a fivefold increase in the counting rate of NRS of SR by ^{174}Yb is achieved. As a result, the spectrum of YbB_{12} can be measured within 10 hours without ^{174}Yb enrichment. Because ^{174}Yb has the largest natural abundance of Yb nuclides (31.8%), a specially enriched sample does not need to be prepared for Yb Mössbauer spectroscopy. The Mössbauer spectrum in the energy-domain is applicable to complex materials. Hence, our system should allow for efficient measurements in a wide variety of scientific areas using the various nuclides including ^{174}Yb .

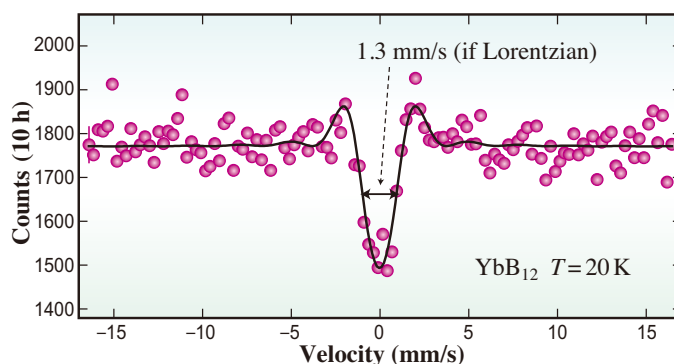


Fig. 3. ^{174}Yb SR-based Mössbauer spectrum of YbB_{12} . Circles and curved line are experimental data and fit using the formulae in Ref. 1, respectively.

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References

- [1] M. Seto *et al.*: Phys. Rev. Lett. **102** (2009) 217602.
- [2] T. Matsuoka *et al.*: Phys. Rev. Lett. **107** (2011) 025501.
- [3] R. Masuda, Y. Kobayashi, S. Kitao, M. Kurokuzu, M. Saito, Y. Yoda, T. Mitsui, F. Iga and M. Seto: Appl. Phys. Lett. **104** (2014) 082411.
- [4] G.K. Shenoy and F.E. Wagner: Mössbauer Isomer Shifts (North-Holland, Amsterdam, 1978) p. 711.
- [5] F. Iga *et al.*: J. Magn. Magn. Mater. **177-181** (1998) 337.

Pt chemical species mapping in polymer electrolyte fuel cells by spatially-resolved nano-XAFS techniques

Polymer electrolyte fuel cells (PEFCs) are one of clean energy-converting devices with high power densities and efficiencies [1]. For widespread commercialization of PEFC vehicles, improvements in reliability and durability are indispensable. To solve these problems, testing and diagnostic methods, which can validate the membrane electrode assembly (MEA) catalyst designs and prove fundamental issues for development of next-generation PEFCs, are mandatory. Particularly, it is necessary to clarify key factors and mechanisms that promote or degrade performances of the Pt/C cathode catalyst and improve the durability for oxygen reduction reactions (ORRs). Key reaction processes, which regulate the durability of PEFCs as well as the ORR activity, may occur heterogeneously in the space of the cathode layer due to the following reasons: (1) the spatially heterogeneous property, (2) the distribution of Pt nanoparticles and carbon supports, and (3) the microscopically non-uniform potentials loaded in the cathode layer under the PEFC potential operations [2]. The cathode degradation mechanism has also been studied to improve the MEA durability, which is a serious issue. Therefore, the nanoscopic spatial place and mechanism of the dissolution and deterioration of the Pt/C cathode catalysts in MEAs should be elucidated to develop next-generation PEFCs with a high durability. Hence, we have investigated these properties at the new high-performance XAFS beamline BL36XU [3].

We prepared two types of MEAs as typical examples: (i) a MEA with a flat interface and few microcracks (denoted as MEA-A) and (ii) a MEA with microcracks (denoted as MEA-B) for nano-XAFS measurements. These MEAs were electrochemically aged and subjected an accelerated durability test (ADT). The treated MEAs were sliced into small pieces for nano-XAFS measurements under humid N_2 (Fig. 1). Because the samples are equivalent to MEAs *in situ* under the aging and ADT, the resultant nano-XAFS spectra are regarded as equivalent to the nano-XAFS spectra measured *in situ* after the aging and ADT cycles. We performed the scanning nano-XAFS mappings and nano-QXAFS measurements with nano-beams of 570 nm $\times$ 540 nm [4].

Figure 2 shows the scanning nano Pt L_{III} -edge XANES mapping for MEA-A before (A and B) and after the ADT cycles (a and b). In the aged MEA-A, the Pt valence map (B) in the whole cathode area does not change significantly. The line profiles of the Pt quantity (blue) and Pt valence (red) along the red arrow of Fig. 2(B) are shown in Fig. 2(C). The Pt valence is metallic

in the whole region, while the distribution of Pt in MEA-A after the ADT cycles is heterogeneous (Fig. 2(a)). The line profiles of the Pt quantity (blue) and Pt valence (red) along the red arrow of Fig. 2(b) are shown in Fig. 2(c). It is noteworthy that the Pt valence in the cathode layer begins to increase at a distance roughly 3.2 μ m away from the cathode layer edge and shows a maximum between the electrolyte and the Pt band (Fig. 2(d)). The oxidized Pt species produced initially at the boundary of the cathode catalyst layer during the operating process involving repeated potential loads.

Figure 3 exhibits the Pt L_{III} -edge jump mapping (A) and Pt valence mapping (B) around the Pt/C cathode layer with a microcrack in MEA-B after the ADT cycles. The microcrack region shows much higher white line peak area (WLPA) values than the other cathode areas. It is noteworthy that the Pt valence in most parts of the microcrack region is calculated as 2+, whereas Pt nanoparticles in the other cathode areas are zero valent (metallic). In the microcrack region, Pt nanoparticles/nanoclusters are not observed by TEM, indicating that the microcrack region contains only sub-nanosized species and/or Pt ions. Figure 3(C) shows the nano-QEXAFS Fourier transforms (black), curve-fittings (red) and determined structural parameters for each area 1–6 in the microcrack region. Microcrack areas 1, 5, and 6 accommodate Pt^{2+} species and the coordination numbers (CN) of Pt-O is 4.0 ($\pm$ 0.4) but Pt-Pt bonding is not observed. This means that the Pt^{2+} species does not possess any Pt-Pt bonds but has a Pt^{2+} -O₄ structure.

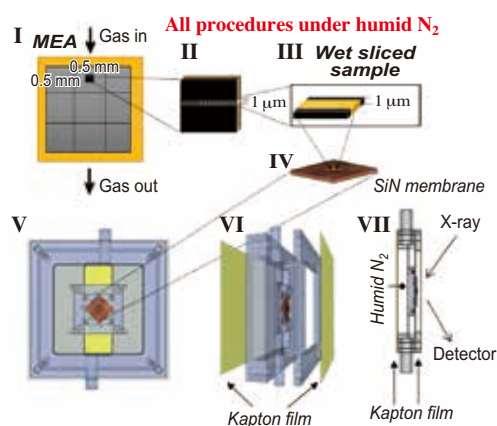


Fig. 1. Preparation and arrangement of a sliced MEA piece on a SiN membrane and its mounting in a XAFS cell for the nano-XAFS measurements. (I) Location of sample extraction, (II and III) dimensions for sample slicing, (IV) mounting on a SiN membrane, (V) front view of the designed cell, (VI) side view of stacked cells, and (VII) directions of the X-ray irradiation and XAFS detector.

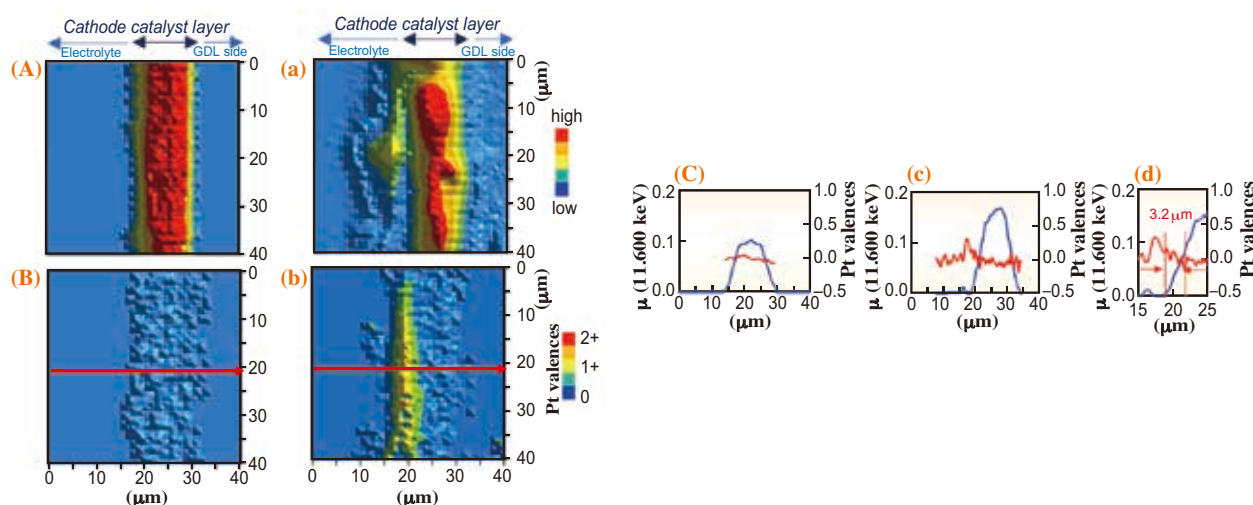


Fig. 2. (A and a) Pt L_{III} -edge jump mapping (Pt quantity mapping), (B and b) Pt valence mapping, for MEA-A after the aging (A, B) and ADT cycles (a, b) by the scanning nano (570 nm $\times$ 540 nm)-XAFS mapping method. (C and c) Line profiles of the absorbance (μ) at 11.600 keV (blue) and Pt valence (red) in the scanning nano-XANES spectra along the red arrows in B and b, respectively. (d) Enlarged line profile around the interface of the cathode catalyst layer and electrolyte.

In conclusion the present nano-XAFS study on the MEAs reports the first successful mapping of the Pt valence and identifies the major locations for oxidation and dissolution of Pt nanoparticles by the ADT cycles. The first spatial approaches to the Pt/C cathode catalysts in PEFCs by the scanning nano-XAFS mapping method and the nano-QXAFS method provide insight into the two-dimensional depth distribution of the metallic and

oxidized Pt species as well as the site-preferential mechanism for Pt oxidation and dissolution to form the Pt^{2+} monomeric species with a $Pt-O_4$ coordination structure in the degradation process. *In situ* nano-XAFS experiments may provide a more in depth understanding of the MEA degradation mechanism by time-dependent mapping of the chemical change of Pt nanoparticles in PEFC MEA under various conditions.

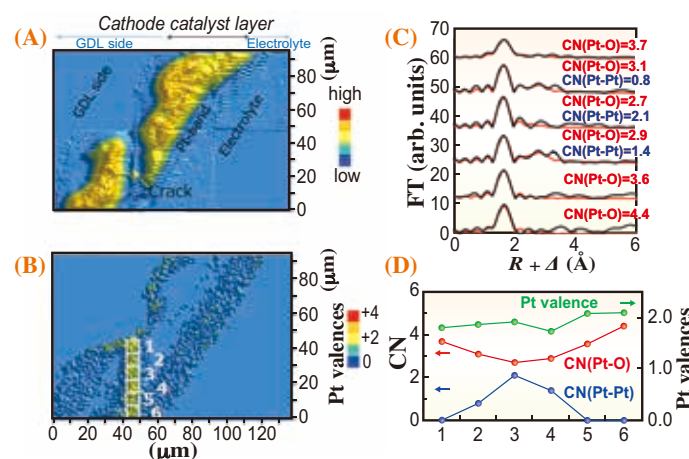


Fig. 3. (A) Pt L_{III} -edge jump mapping (Pt quantity mapping), (B) Pt valence mapping for MEA-B after the ADT cycles by the scanning nano-XAFS mapping method. (C) Nano-QEXAFS Fourier transforms (black) and curve-fittings (red) for microcrack areas (1-6) in B. (D) Nano QEXAFS analysis (CN(Pt-Pt):blue, CN(Pt-O):red) and Pt valence (green) for each microcrack area (1-6) in B.

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References

- [1] G. Wu *et al.*: Science **332** (2011) 443; M.K. Debe: Nature **486** (2012) 43.
- [2] T. Saida *et al.*: Angew. Chem. Int. Ed. **51** (2012) 10311.
- [3] S. Takao, O. Sekizawa, S. Nagamatsu, T. Kaneko, T. Yamamoto, G. Samjeske, K. Higashi, K. Nagasawa, T. Tsuji, M. Suzuki, N. Kawamura, M. Mizumaki, T. Uruga, Y. Iwasawa: Angew. Chem. Int. Ed. **53** (2014) 14110.
- [4] O. Sekizawa *et al.*: J. Phys. Conf. Ser. **430** (2013) 012020.

In situ observations of fuel cell electrodes by ambient-pressure hard X-ray photoelectron spectroscopy

The polymer electrolyte fuel cell (PEFC) is a promising technology as a highly-efficient clean energy source for automobiles. Many issues, however, remain to be solved, such as the improvement of power generation performance and the reduction in the usage of expensive platinum catalysts in the cathode. The development of next-generation high-efficiency fuel cells requires that the oxygen reduction reaction mechanism, which occurs at the cathode catalysts, to be well understood (Fig. 1). Direct observations of the electronic states in the catalyst materials under fuel-cell conditions should help realize a highly active catalyst in membrane electrode assemblies (MEAs) under fuel-cell reaction conditions.

X-ray photoelectron spectroscopy (XPS) is a powerful technique to investigate electronic states, but the application of XPS at elevated pressures is complicated due to two factors. One is the necessity of low pressures in the electron energy analyzer to avoid discharges on the electrostatic lens elements and the detectors. The other is attenuation of photoelectrons through the gases in the measurement chamber due to the strong photoelectron interactions with gaseous molecules at energies typically used in XPS. The first factor was overcome by long differential pumping system with a small aperture to reduce the molecular flows into the analyzer, whereas the latter was resolved through a shorter working distance between the sample surface and the aperture. Recently, the development of photoelectron spectrometers and the use of intense X-rays from synchrotron radiation sources have allowed XPS spectra to be measured under near ambient pressure conditions. This technique is known as ambient pressure X-ray photoelectron spectroscopy (AP-XPS) [1].

Meanwhile, XPS using hard X-rays (HAXPES) was developed during the last decade to observe buried samples [2]. One of the advantages of HAXPES is a long mean free path of photoelectrons when their kinetic energy is increased. If a beam energy in the hard X-ray region is used in AP-XPS, then spectra can be obtained under higher pressures than those in the case of soft X-rays. Moreover, it becomes possible to observe liquid/solid interfaces in heterogeneous catalysts and buried nanoparticles in a polymer electrolyte membrane because the inelastic mean free path of electrons within a solid, which has a kinetic energy of 8 keV, is 5–50 nm. A system that combines AP-XPS and HAXPES, i.e., ambient pressure hard X-ray photoelectron spectroscopy (AP-HAXPES),

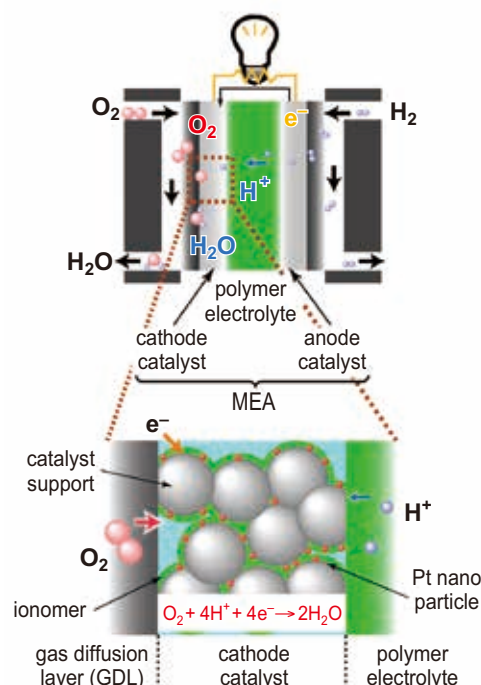


Fig. 1. Schematic drawing of PEFC.

should facilitate the observation of the electronic states of catalysts at buried interfaces under reaction conditions.

We developed a near ambient pressure photoelectron spectroscopy instrument that is used with hard X-rays at beamline BL36XU (Catalytic Reaction Dynamics for Fuel Cells) [3]. The experiments were conducted using a commercial differential-pumping type analyzer (R4000 HiPP-2, VG Scienta) [4]. The equipment consists of a pre-lens in the differential pumping chamber evacuated with several vacuum pumps and a standard hemispherical electron energy analyzer. Using the front cone with a 300- μm aperture diameter, the pressure of the measurement chamber can be increased up to 3,000 Pa during operation.

The Au 4f spectra obtained at an incident photon energy of 7.94 keV at various nitrogen gas (N_2) pressures are shown in Fig. 2. The intensity of the spectra is almost constant under the conditions from the high-vacuum (2×10^{-5} Pa) to 100 Pa, but decreased rapidly as the N_2 pressure is increased to 3,000 Pa due to photoelectrons scattering by gaseous molecules. The signal attenuation from the signal level in the high vacuum is, however, only 46% at 3,000 Pa,

which indicates that the apparatus allows AP-HAXPES measurements to be performed at the saturation vapor pressure of water at room temperature.

We performed *in situ* AP-HAXPES measurements of the Pt/C cathode of the PEFC [5]. To observe the modified electronic states of Pt atoms in Pt/C cathode catalysts during operations, the Pt $3d_{5/2}$ peaks were recorded at an incident X-ray beam energy of 7.94 keV (Fig. 3). We performed curve fitting analysis for these peaks with three components at 2122.0, 2123.3, and 2125.2 eV. The lowest energy peak is assigned to Pt metal (Pt^0) and the highest one to Pt^{2+} species such as PtO. The peak at 2123.3 eV is attributed to Pt^{1+} species such as PtOH.

When the cathode is exposed to H_2O and hydrogen gas flowed in the anode side under the open circuit condition, a voltage of 0.12 V is generated between the electrodes. The spectrum shows a major metallic Pt peak at 2122.0 eV, along with small peaks at 2123.3 eV from the surface Pt atoms with contaminants (Fig. 3(a)). When the applied voltage is maintained at 1.3 V, the higher-energy peak at 2125.2 eV for PtO was observed, in addition to the peak 2123.3 eV (Fig. 3(b)). In contrast, the peak at 2125.2 eV almost disappears when the applied voltage is decreased to 0.1 V (Fig. 3(c)). The Pt nanoparticles at the cathode are oxidized and reduced when the applied voltage between the electrodes was increased to greater than 0.8 V and decreased to less than 0.7 V, respectively. The observed peaks reflect oxidized Pt nanoparticles with different valences on the MEA electrode, depending on the applied voltage. Consequently, the AP-HAXPES

apparatus allows the oxidization/reduction reactions of the electrodes to be observed during the operation of a fuel cell, promoting the development of fuel cell electrodes and catalyst materials.

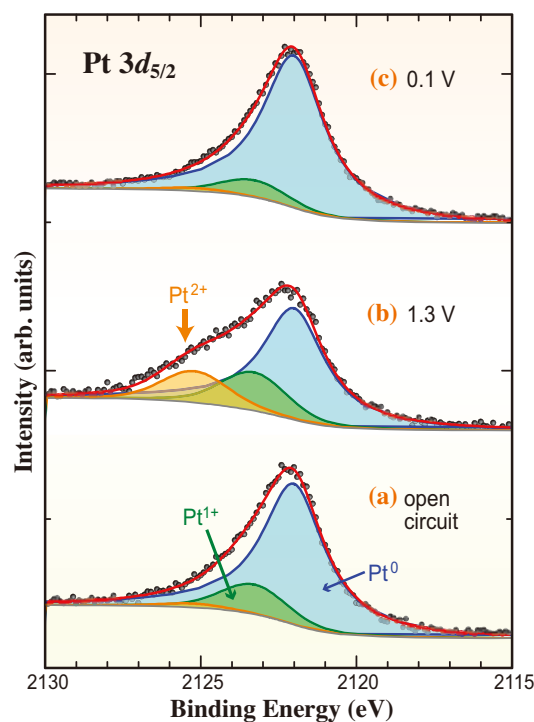


Fig. 3. *In situ* AP-HAXPES Pt $3d_{5/2}$ spectra of the Pt/C catalyst in the cathode.

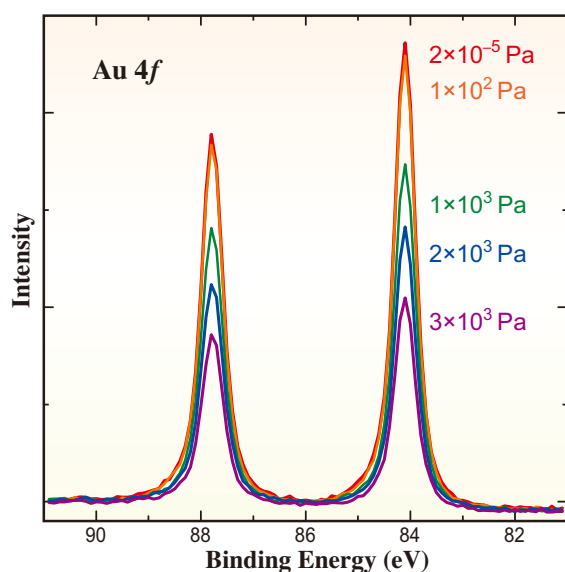


Fig. 2. Au $4f$ spectra of Au foil recorded with a 7.94 keV photon energy under different pressures of N_2 .

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References

- [1] D.F. Ogletree *et al.*: Rev. Sci. Instrum. **73** (2002) 3872.
- [2] Y. Takata *et al.*: Nucl. Instrum. Meth. Phys. Res. Sect. A **547** (2005) 50.
- [3] O. Sekizawa *et al.*: J. Phys.: Conf. Ser. **430** (2013) 012020.
- [4] S.K. Eriksson *et al.*: Rev. Sci. Instrum. **85** (2014) 075119.
- [5] Y. Takagi, H. Wang, Y. Uemura, E. Ikenaga, O. Sekizawa, T. Uruga, H. Ohashi, Y. Senba, H. Yumoto, H. Yamazaki, S. Goto, M. Tada, Y. Iwasawa and T. Yokoyama: App. Phys. Lett. **105** (2014) 131602.

Remarkably enhanced hydrogen-storage capacity and speed in Pd nanocrystals covered with a metal-organic framework

Hydrogen is an essential component in many industrial processes. Due to the recent shale gas revolution and the need to use hydrogen more effectively, hydrogen production through steam reforming reaction of methane has received increased attention. Palladium has been intensively researched for hydrogen storage and hydrogen-related catalytic reactions because hydrogen easily dissociates on the surface of Pd, and hydrogen atoms can permeate into the metal lattice. To date, attempts to improve the hydrogen storage properties of Pd have typically involved the creation of Pd alloys [1]. Metal-organic frameworks (MOFs), which are assembled from metal ions and organic bridging ligands, are promising as coatings on Pd for more effective hydrogen storage due to their high surface area, porosity, and the condensation effect. Here, we introduce the remarkably enhanced capacity and speed of hydrogen storage in Pd nanocrystals as a result of coating with a MOF, copper(II) 1,3,5-benzenetricarboxylate ($\text{Cu}_3(\text{BTC})_2$, [2] HKUST-1).

We synthesized Pd nanocrystals coated with HKUST-1 (Pd@HKUST-1) by a facile reactive seeding method, where the Pd nanocrystals act as seeding sites for MOF growth [3]. The powder X-ray diffraction (XRD) pattern of the composite consists of diffracted peaks from both the Pd and HKUST-1 lattices, indicating that the composite includes both Pd and HKUST-1. From the transmission electron microscopy (TEM) images of the Pd nanocubes and the composite (Figs. 1(a) and 1(b)), the MOF film was observed around the Pd nanocubes after hybridization. The scanning TEM-energy-dispersive X-ray maps demonstrated that the obtained material forms Pd nanocrystals covered with HKUST-1 [3].

The hydrogen storage properties of Pd@HKUST-1 were investigated by hydrogen pressure-composition isotherm measurements at 303 K, as shown in Fig. 1(c). The hydrogen concentration in Pd increases with increasing hydrogen pressure for both materials, but the total amount of hydrogen absorption at 101.3 kPa was enhanced from 0.5 H/Pd in bare Pd nanocubes to 0.87 H/Pd for the Pd@HKUST-1. Considering that pure HKUST-1 does not adsorb H_2 at all, the absorption of 74% more hydrogen is attributed to the Pd nanocubes within the Pd@HKUST-1.

We elucidated the structural change during the hydrogen absorption/desorption process by *in situ* XRD measurements every 10 min for each hydrogen pressure at BL02B2 beamline (Figs. 2(a) and 2(b)).

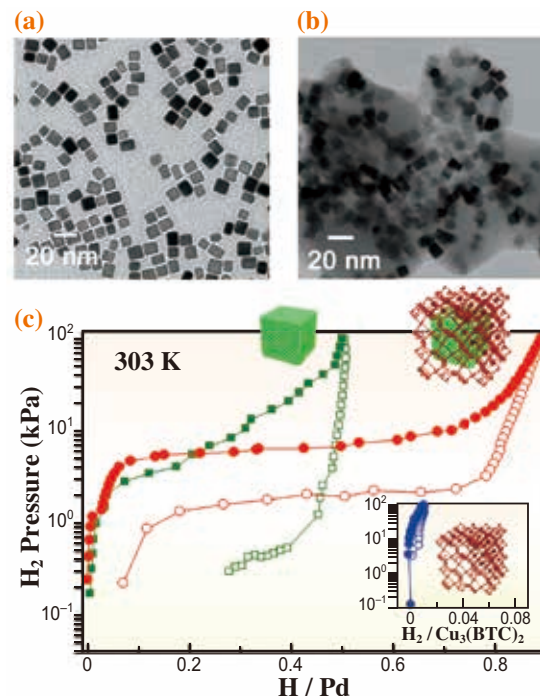


Fig. 1. TEM images of (a) Pd nanocubes and (b) Pd@HKUST-1. (c) Pressure-composition isotherms of Pd nanocubes (green) and Pd@HKUST-1 (red) at 303 K.

In addition to the unchanged diffraction from the α phase solid solution Pd lattice, the diffraction peaks for the bare Pd nanocubes from the face-centered-cubic (fcc) hydride β phase lattice began to appear at the lower-angle side during hydrogen absorption. Upon further increasing the hydrogen pressure, these two fcc components remained, even in the hydrogen desorption process at 0 kPa. On the other hand, in Pd@HKUST-1, the diffraction peaks completely shifted to the lower-angle side with hydrogen pressure and then returned to the same positions as the pristine sample upon the desorption process. The reversible hydrogen absorption/desorption behavior of Pd@HKUST-1 suggests that the absorption/desorption response of Pd nanocrystals is enhanced by the HKUST-1 coating. To obtain direct evidence of the enhanced hydrogen storage speed for the Pd nanocubes coated with HKUST-1, the kinetics of the hydrogenation of Pd nanocubes and Pd@HKUST-1 were investigated using the isothermal hydrogenation profiles at 303 K after introducing a hydrogen pressure of 101.3 kPa, as shown in Fig. 2(c). Within 10 min the Pd nanocubes absorbed only 0.11 H/Pd (ca. 22% of total capacity), while Pd@HKUST-1 absorbed a significant amount of

hydrogen, 0.33 H/Pd (ca. 38% of the total capacity). These results indicate that Pd@HKUST-1 can absorb hydrogen about twice as fast as the Pd nanocubes within the first 10 min.

The change in the electronic structure due to the HKUST-1 coating on the surface of Pd was also investigated by X-ray photoelectron spectroscopy (XPS) measurements. The XPS spectra of Pd@HKUST-1 suggested that the electronic states of Pd@HKUST-1 differ from those of bare Pd nanocubes and pure HKUST-1, and that the electrons in the Pd nanocubes are slightly transferred to the HKUST-1 (Fig. 3). It has been reported for Pd alloys that the hydrogen concentration in Pd increases with the number of 4d-band holes and that there is a relationship between band filling and the hydrogen concentration [4]. This electron transfer may be responsible for the increased number of holes in the 4d band of Pd nanocubes with the HKUST-1 coating and the origin of the enhanced hydrogen storage capacity.

Our present work suggests that the presence of a MOF coating significantly altered the surface/bulk reactivity of the nanocrystals, allowing for a more facile reactivity with hydrogen. Although Pd is relatively scarce and expensive, this method of enhancement may be

utilized to bolster the hydrogen storage properties using less expensive metals. This enhanced reactivity may also be applicable to other metal@MOF systems, possibly providing a general method to improve the reactivity of nanocrystals.

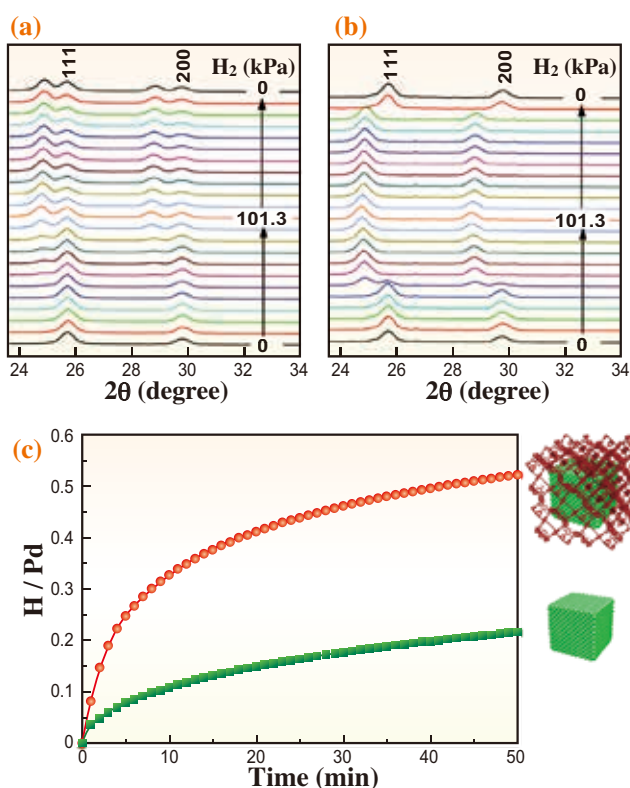


Fig. 2. *In situ* powder XRD patterns of (a) Pd nanocubes and (b) Pd@HKUST-1 upon the process of hydrogen absorption/desorption at 303 K. (c) Isothermal hydrogenation profiles of Pd nanocubes (green) and Pd@HKUST-1 (red) at 303 K after introducing a hydrogen pressure of 101.3 kPa.

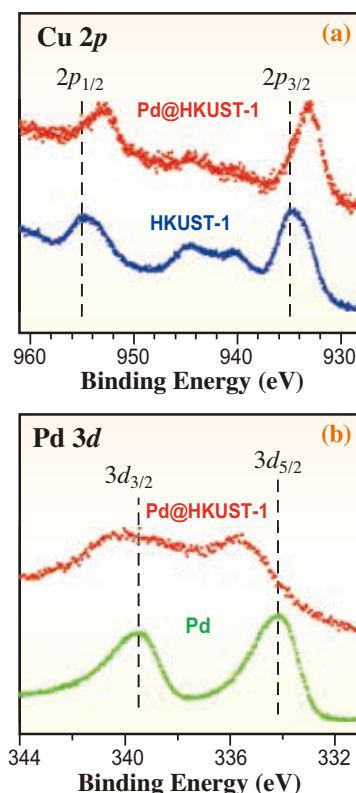


Fig. 3. XPS spectra of (a) Cu 2p and (b) Pd 3d (green : Pd nanocubes; blue : HKUST-1; red : Pd@HKUST-1).

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References

- [1] G. Alefeld and J. Völkl: Hydrogen in Metals II (Springer, Berlin, Heidelberg, 1978).
- [2] S. S.-Y. Chui *et al.*: Science **283** (1999) 1148.
- [3] G. Li, H. Kobayashi, J.M. Taylor, R. Ikeda, Y. Kubota, K. Kato, M. Takata, T. Yamamoto, S. Toh, S. Matsumura and H. Kitagawa: Nat. Mater. **13** (2014) 802.
- [4] D.A. Papaconstantopoulos *et al.*: Phys. Rev. B **17** (1978) 141.

Inhomogeneity of liquid water revealed by resonant soft X-ray vibrational spectroscopy

The unique properties of water, e.g., high boiling and melting points compared to molecules like non-metal hydrides and its reduced density of the solid form are explained by the attractive force between water molecules called hydrogen bonds. There are many proposed local structural models that describe a water network. Among them two are well known: the continuum model and the mixture (micro-heterogeneity) model. In the former, hydrogen bonds in water are distorted, broken, and reformed continuously, but water itself is composed of a single component, whereas in the latter, the network is considered as a mixture of various hydrogen bond configurations. However, which model better describes the hydrogen bonding property of liquid water is controversial.

X-ray or neutron diffraction is one of the best methods to reveal local structural information of liquid water. When combined with recent highly brilliant synchrotron sources, not only the distance between the nearest neighbors but also those distances several water molecules apart can be discussed by diffraction techniques [1]. However, these methods collect signals mostly from scattering of an X-ray by a core electron or of a neutron by atomic nucleus, and thus indirectly reflect information about hydrogen bonds. Consequently, X-ray spectroscopies have attracted much attention in this decade as alternatives to diffractions since they provide direct information about hydrogen bonds through observations of the valence electronic structure.

One of the most famous works was performed by Wernet *et al.* in 2004 [2]. They focused on the O 1s X-ray absorption (XAS) profile of water in the gaseous, liquid, and solid phases (Fig. 1 [3]). They concluded that the XAS pre-edge peak represents a hydrogen bond breaking and the number of hydrogen bonds in liquid water is much less than generally considered. Their interpretation has been challenged by many experimental and theoretical works. In particular, the presence of the XAS pre-edge peak has been interpreted not as the hydrogen bond breaking but distortions that leave the hydrogen bond intact. The interpretation of the XAS pre-edge peak is quite important since the hydrogen-bond-broken and hydrogen-bond-intact pictures lead to the mixture (micro-heterogeneity) and the continuum models of liquid water, respectively.

To clarify the origin of the XAS pre-edge, we applied resonant soft X-ray vibrational spectroscopy, which leaves only vibrational excitations in the soft X-ray Raman process (Fig. 2). The resonant excitation of the core electron to a specific unoccupied state

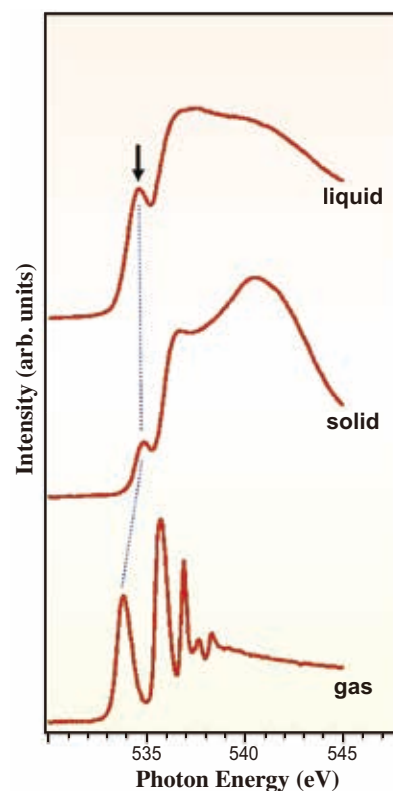


Fig. 1. O 1s X-ray absorption profile of water in the liquid, solid, and gas phases. Arrow in the XAS spectrum of liquid water indicates the pre-edge structure.

should relate the observed energies without electronic excitation to the vibrational excitations of a water molecule in a particular hydrogen bond configuration. The experiments were carried out using high resolution X-ray emission spectrometers at University-of-Tokyo Synchrotron Radiation Outstation beamline **BL07LSU** and RIKEN Coherent Soft X-ray Spectroscopy beamline **BL17SU**.

Figure 3(a) shows the vibrational excitations through O 1s resonant excitation to the XAS pre-edge of liquid H₂O and D₂O water [4]. The high resolution ($E/\Delta E > 2000$) reveals isolated multiple peak structures. The energy separation of the corresponding peaks in H₂O from the elastic line is $\sqrt{2}$ times larger than those in D₂O, reflecting an isotope effect on the OH stretching energy. Owing to the strong dissociation potential in the presence of an excited electron in the antibonding state, which is localized on one OH (OD) lobe, multiple peak structures extend over the energy window between the valence band maximum around 527 eV and the

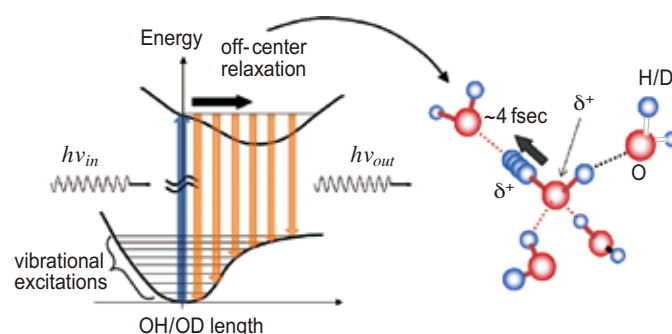


Fig. 2. Schematic of the soft X-ray Raman process that leaves vibrational excitations. In the core excited state, ultrafast dissociation occurs within the lifetime of the core hole (~ 4 fs) due to the presence of core excited electron in the antibonding orbital on one of the OH (OD) lobes, which facilitates elongation of the OH (OD) bond as illustrated in the right panel.

elastic line at 535 eV. The energy separation of the neighboring peaks, which decreases at higher order vibrational excitations (Fig. 3(b)), can be explained by a Morse function.

What should be noted here is the absolute value of the first vibrational energy. In both H_2O and D_2O , the value is very close to the OH (OD) stretching vibrational energy of an isolated water molecule estimated from

the optical Raman experiment (H_2O : 0.45–0.46 eV, D_2O : 0.33–0.34 eV), but it is slightly (~ 30 meV) higher than the centroid of the OH(OD) stretch band of liquid water (H_2O : 0.42 eV, D_2O : 0.31 eV), as shown in Fig. 3(b). This is clear evidence that the XAS pre-edge peak mainly originates from a water molecule in a broken hydrogen bond configuration, and thus supports the mixture (micro-heterogeneity) model of liquid water. This is also in accordance with the interpretation of our previous X-ray emission results on liquid water reported since 2008 [5].

As demonstrated in this study, core spectroscopy of liquid water is now connected to vibrational Raman spectroscopy, and can clarify the hydration structure, the role of water in various chemical and catalytic reactions, and hydrogen bonds in biological organisms.

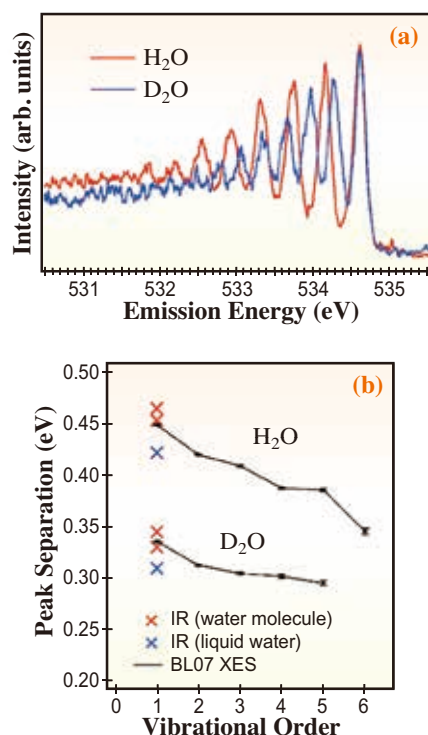


Fig. 3. (a) Comparison of multiple vibrational excitation spectra between H_2O and D_2O . (b) Energy separation of each neighboring peak in the resonant soft X-ray vibrational spectrum of H_2O and D_2O . Red and blue crosses denote the OH stretching energy estimated from Raman spectra of gaseous and liquid water, respectively.

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References

- [1] C. Huang *et al.*: Phys. Chem. Chem. Phys. **13** (2011) 19997.
- [2] P. Wernet *et al.*: Science **304** (2004) 995.
- [3] S. Myneni *et al.*: J. Phys.: Condens. Matter **14** (2002) L213.
- [4] Y. Harada, T. Tokushima, Y. Horikawa, O. Takahashi, H. Niwa, M. Kobayashi, M. Oshima, Y. Senba, H. Ohashi, K.T. Wikfeldt, A. Nilsson, L.G.M. Pettersson and S. Shin: Phys. Rev. Lett. **111** (2013) 193001.
- [5] T. Tokushima *et al.*: Chem. Phys. Lett. **460** (2008) 387.

Dynamical active optics for hard X-ray lasers

Many functional laser devices have been developed for optical lasers. Better quality light is obtained with these devices, and now many applications use well controlled lasers. The history of optical laser science is a story of improving control of the light. The same approach is expected for hard X-ray lasers. After successful generation of X-ray lasers from a free electron laser facility [1,2], these expectations are increased. Today we see the production of well-tuned high-energy photons having high spatial coherence which can be focused to high intensity. These features will powerfully advance X-ray science. The results described here demonstrate a start of these developments for hard X-ray laser research.

In normal active optical devices, the propagation constants such as the optical refractive index are changed and/or modulated by light field itself or by applied electric or magnetic fields. The mechanisms include the Pockels effect, Kerr effect, self-phase modulation, self-focusing, and other nonlinear scattering. They change the light direction, temporal shape of the pulse, wavelength and so on. In the X-ray region, the fundamental optical constants (real and imaginary parts of the refractive index, $n-1$ and k) are much smaller than those at optical wavelengths. That means that the nonlinear coefficient is very small for hard X-rays so that higher intensity is needed to produce nonlinear effects. In this situation, we proposed to use the “K-edge absorption resonance” because that gives the largest change of optical parameters for hard X-rays. As shown in Fig. 1, the edge energy is shifted with ionization of a 1s electron. If the edge energy changes, we expect a large change in the optical constants.

The intensity required to produce and maintain a single vacancy in the K-shell in a solid is determined by the photo-absorption cross section and by the speed of refilling of the K-shell vacancy by L- and M-shell electrons. When we consider the photon energy of the present beamline in the SACLA facility, the candidate atoms are medium Z atoms from Ti (5 keV) to Zn (10 keV). The critical intensity for those atoms is several times 10^{19} W/cm². This X-ray intensity has been achieved for the first time in the newest two stage focusing system in the SACLA facility [3].

The first experiment for developing active optics in the hard X-ray range was performed at the EH5 station of the BL3 beamline where a 50 nm focusing optics can be used [4]. We selected iron as a target material. The XFEL is tuned to the iron K-edge absorption (7.1 keV). The transmitted FEL X-rays are measured with a crystal spectrometer with 1D spatial resolution. Figure 2 shows intensity dependence of transmission of 7.13 keV X-ray. One order of magnitude increase of transmission is observed at $I = 7 \times 10^{19}$ W/cm². The change of the absorption starts at $I = 10^{19}$ W/cm² and this number agrees with the predicted intensity based on a rate comparison between photo-ionization and Auger process. This result shows that we can change the imaginary part of the refractive index with an intense hard X-ray laser pulse. Due to the Kramers-Kronig relation, the real part of refractive index also changes in this high intensity interaction region. If we change the real part of refractive index with the intense X-ray laser, the spatial profile of the transmitted light should be changed. To see that, we examine the divergence profile of the transmitted pulse. In the

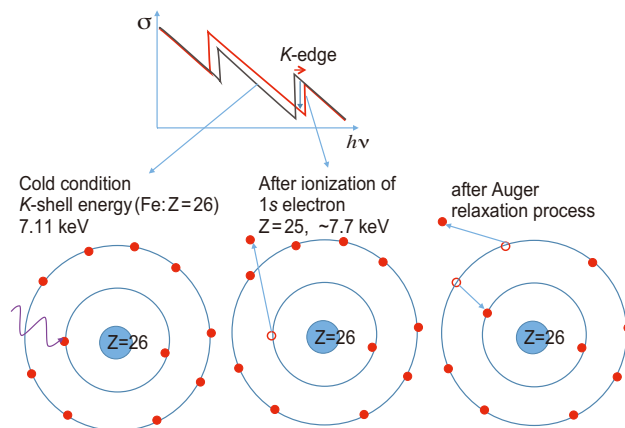


Fig. 1. The absorption edge spectrum changes with photoionization. After removal of one K-shell electron, the effective binding energy of the remaining 1s electron will increase by about 0.6 keV. This shift causes a relatively large transmission change.

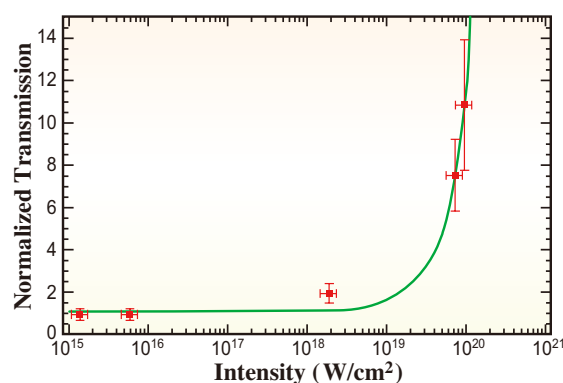


Fig. 2. Transmission normalized at the cold condition is plotted as a function of intensity at $h\nu = 7.130$ eV for 20- μm -thick iron foil. The square dots show the experimental results, while the green lines are simulated curves with core-hole lifetimes of 2 fs.

experiment, we observe that the divergence angle of the transmission X-ray changes as shown in Fig. 3. At low intensity, the transmitted XFEL has 4 mrad expansion divergence, determined by the numerical aperture for the final focusing optics. At the highest intensity and for $h\nu > 7.13$ keV, this angle decreases to 2.3 mrad. That means dynamical spatial filtering occurs in the nonlinear optical interaction process. Similar mode cleaning is observed in saturable absorbers in the optical frequency region. To our knowledge, this

is the first observation of this phenomenon in the hard X-ray region.

In conclusion, nonlinear transmission of X-rays is clearly observed in the multi-keV X-ray region for the first time. The threshold intensity for this nonlinear transmission agrees with the estimated value at which the rate of the photoionization equals the Auger rate. Intensity-dependent spatial filtering is also observed. These nonlinear transmission processes will be used for an ultra-fast X-ray switch in near future.

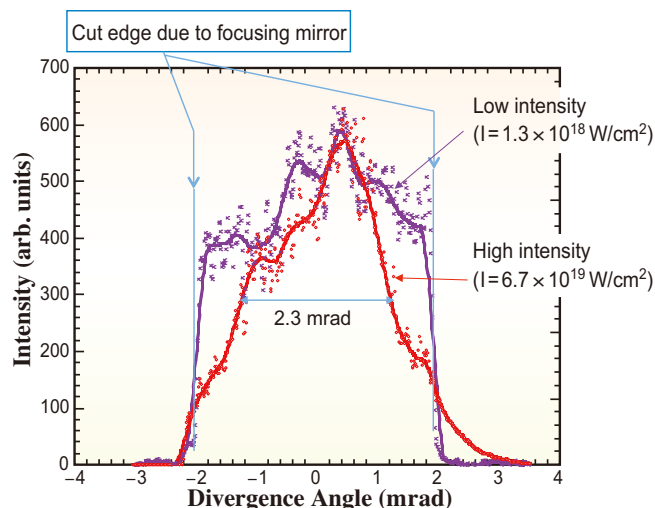


Fig. 3. Profile of angular divergence of the transmitted laser pulse. The red line shows high intensity results while the violet line shows a lower intensity case. The divergence of the beam incident on the target is limited by the acceptance angle of the final optics. The transmitted light has smaller divergence and a Gaussian-like profile.

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References

- [1] P. Emma *et al.*: Nat. Photonics **4** (2010) 641.
- [2] T. Ishikawa *et al.*: Nat. Photonics **6** (2012) 540.
- [3] H. Mimura *et al.*: Nat. Commun. **5** (2014) 3539.
- [4] H. Yoneda, Y. Inubushi, M. Yabashi, T. Katayama, T. Ishikawa, H. Ohashi, H. Yumoto, K. Yamauchi, H. Mimura and H. Kitamura: Nat. Commun. **5** (2014) 5080.

X-ray two-photon absorption

Two-photon absorption (TPA) is one of the most fundamental nonlinear optical phenomena. Although it is widely used for microscopy and spectroscopy in the visible region, it has yet to be observed in the hard X-ray region. In the TPA process, the two photons are absorbed simultaneously, because there is no real intermediate state. When we consider TPA near the threshold, i.e., the pump photon energy is just above the half of the absorption edge, the lifetime of the virtual intermediate state can be approximated by $1/\omega$, where ω is the pump frequency, and the TPA cross section may be written as [1]:

$$\sigma_{TPA} = \sigma \frac{1}{\omega} \sigma'$$

where σ and σ' are the cross sections for the one-photon absorption (OPA). Since TPA consists of two OPA processes, the selection rule is expected to be different from OPA. The OPA process is allowed between states with different parities, e.g., $1s$ and $4p$, while TPA is between states with the same parity, e.g., $1s$ and $3d$. This unique nature of X-ray TPA should realize a new kind of X-ray spectroscopy, namely, X-ray TPA spectroscopy.

Here, we roughly estimate σ_{TPA} in the X-ray region. The OPA cross-sections and the frequency are typically $\sigma, \sigma' \sim 10^{-20} \text{ cm}^2$ and $\omega \sim 10^{-19} \text{ 1/s}$, resulting in a very small TPA cross-section of $\sigma_{TPA} \sim 10^{-59} \text{ cm}^4/\text{s}$. The TPA process is comparable to the OPA process, i.e., $\sigma \sim I\sigma_{TPA}$, when the peak intensity reaches $I \sim 10^{39}$ photons/cm²s. Such a high intensity can be realized by an X-ray pulse with a flux of 10^{14} photons and 1-fs

pulse duration focused down to the 100-nm size. Even if we use a state-of-the-art X-ray free-electron laser and focusing technique, the observation of X-ray TPA is still very challenging.

To observe X-ray TPA, we used the 50-nm focusing system installed in the experimental station 5 at beamline BL3 of SACLA [2]. The sample was a thin germanium plate. The photon energy was 5.6 keV, which is just above half of the K -shell binding energy at 11.1 keV. The measured focus size was 110(H) $\times$ 140(V) nm, and the average pulse energy was 13 μJ . Using a pulse duration of 2.5 fs (full width at half maximum) estimated from the double core-hole experiment [3], the estimated peak intensity is $3.4 \times 10^{19} \text{ W/cm}^2$ (3.7×10^{34} photons/cm²s). We measured X-ray fluorescence by TPA [4] because the contribution of TPA to the total absorption is too small to measure directly. X-rays at the fluorescence photon energy were selected using a bent Si(111) crystal in the Johansson geometry, which was followed by an MPCCD (multi-port CCD) detector. The spectrometer suppresses the elastically and inelastically scattered X-rays, allowing the very weak signal of TPA fluorescence to be measured.

Figure 1 shows the histogram of the readout counts from each pixel of the MPCCD during the experiment. The large peak centered at the origin is the null event. Because the probability of measuring an X-ray photon in a particular pixel of MPCCD is less than 10^{-5} , the readout count is proportional to the photon energy of a single photon. Thus, the histogram can be regarded as the spectrum. The conversion coefficient between the readout count and the photon energy was determined

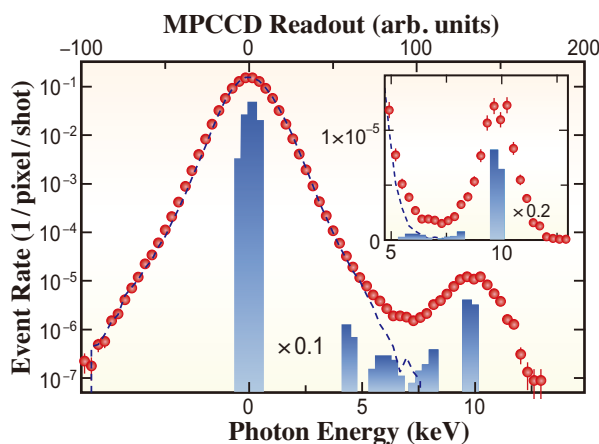


Fig. 1. X-ray fluorescence spectrum of germanium by two-photon absorption [3]. Circles and dashed line denote the measured data and read-out noise spectrum, respectively. Vertical bars indicate the deconvoluted spectrum from the noise, showing a clear fluorescence peak at 10 keV. Inset shows an enlarged spectrum around 10 keV.

experimentally. The width of the peak at the origin represents the photon energy resolution of 1.8 keV. The photon energy of the weaker peak is 10 keV, which corresponds to the germanium fluorescence by TPA.

The germanium fluorescence increases quadratically with the pulse energy (Fig. 2), indicating a two-photon process. However, the fluorescence counts deviate downwards from the quadratic dependence at higher pulse energies. The deviation can be explained as follows. Intense X-rays excite germanium mainly by the OPA process in the *L*-shell. When a core hole is created in the *L*-shell, the *K*-shell binding energy becomes deeper than twice the X-ray photon energy, i.e., 11.2 keV, due to a reduction in the screening effect. Thus, atoms with an *L*-shell core-hole cannot contribute to TPA. The *L*-shell hole decays immediately, leaving two holes in the *M*-shell. Interestingly, an atom with two *M*-shell holes is subject to TPA because the *K*-shell binding energy is less than the TPA threshold.

Since there are many different decay channels, a self-consistent simulation is needed to estimate how many atoms can participate in the TPA process. We solved numerically the coupled rate equations, and

simulated the population dynamics of the electronic configurations of interest [5]. The temporal shape of the X-ray pulse is assumed to be Gaussian, and the spiky time structure is ignored because the lifetimes of the excited states are much longer than the spike duration. The pulse-energy dependence of the TPA fluorescence was calculated based on the time-dependent population dynamics. The unknown parameter in the simulation is the TPA cross section, which is determined by fitting the calculated pulse-energy dependence to the measured data. The best fit is obtained with $\sigma_{TPA} = 6.4 \times 10^{-60} \text{ cm}^4/\text{s}$, which is comparable to the rough estimation above.

In conclusion, we successfully observed X-ray TPA for the first time and estimate the TPA cross section. The different selection rule for TPA from the conventional XAS would be useful for studying the 3*d* orbitals of transition metal compounds. However, the intense X-rays change the electronic structure of the sample within the pulse duration, and the ground-state property is difficult to measure. A more efficient detecting system would allow TPA measurements under moderate peak intensities, enabling X-ray TPA spectroscopy to reveal the ground-state property of a sample.

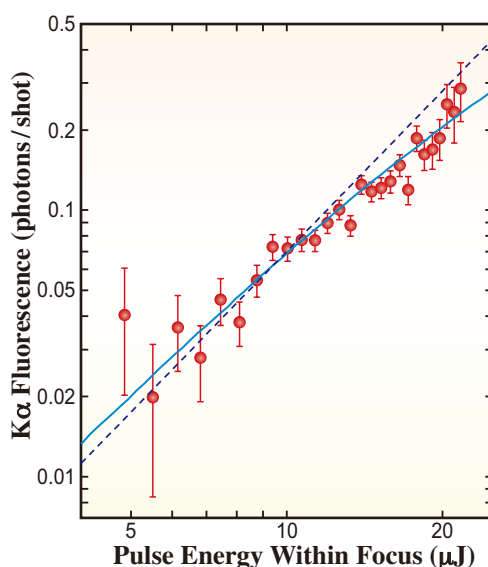


Fig. 2. Pulse-energy dependence of X-ray fluorescence [3]. Circles and vertical bars indicate the measured data and statistical error, respectively. Dashed line shows a simple quadratic dependence with a slope of two, while the solid line is the best fit of the simulation result with the fitting parameter of the TPA cross section.

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References

- [1] P. Lambropoulos and X. Tang: *J. Opt. Soc. Am. B* **4** (1987) 821.
- [2] H. Mimura *et al.*: *Nat. Commun.* **5** (2014) 3539.
- [3] K. Tamasaku *et al.*: *Phys. Rev. Lett.* **111** (2013) 043001.
- [4] K. Tamasaku, E. Shigemasa, Y. Inubushi, T. Katayama, K. Sawada, H. Yumoto, H. Ohashi, H. Mimura, M. Yabashi, K. Yamauchi and T. Ishikawa: *Nat. Photonics* **8** (2014) 313.
- [5] S.-K. Son *et al.*: *Phys. Rev. A* **83** (2011) 033402.

Low core-mantle boundary temperature inferred from the solidus of pyrolite

The Earth's interior can be divided into layers by chemical composition and physical properties (Fig. 1). The interface between the mantle (silicate rock) and the outer core (liquid iron alloy) is located at a depth of 2900 km (136 GPa for pressure). The melting temperature of the mantle provides key constraints on the thermal and chemical structure of the core. The temperature at the top of the core should be lower than the solidus (onset of melting) temperature of a primitive mantle to avoid global melting above the core-mantle boundary (CMB). On the other hand, the temperature on the core side of the CMB should be higher than the liquidus (complete melting) temperature of the liquid outer core. The temperature at the CMB is often believed to be about 4000 K, which is higher than the liquidus temperature of most of the plausible outer core compositions. A laser-heated diamond anvil cell (DAC) can achieve such high pressure and temperature conditions. Previous experiments using laser-heated DAC techniques have shown that the solidus temperature of a primitive mantle is about 4200 K at the CMB [1], supporting a high T_{CMB} around 4000 K. The determination of the solidus temperature using the DAC is, however, challenging because detecting a small amount of partial melt at the onset of melting by X-ray diffuse scattering [1] is difficult, especially for silicates.

We determined the solidus temperature of the lower mantle on the basis of the textural and chemical characterizations of quenched samples after subjecting them to a high-pressure environment like that of the CMB. Our previous melting experiments showed that a partial melt of the mantle material is highly enriched with iron under all mantle pressure conditions [2]. The

3D distribution of iron can be obtained by imaging the sample at X-ray energies of 7 and 8 keV (K -absorption edge of Fe is 7.11 keV) using analytical dual-energy microtomography with a high spatial resolution of ~ 200 nm (pixel size: ~ 70 nm) and a $110\text{-}\mu\text{m}$ field of view under the typical conditions at beamline **BL47XU** [3], which are sufficient to image the internal structure of small DAC samples (typically about $40\text{ }\mu\text{m}$) at the CMB pressure. This suggests that the detection of iron enrichment can be used as a tracer for a tiny partial melt at the onset of melting.

Our starting material possessed a natural primitive mantle (pyrolite) composition with about 400 parts per million (ppm) H_2O . High pressure and temperature experiments were conducted using laser-heated DAC techniques. The starting material was heated to 2100–3900 K at 25–169 GPa in a DAC, which covers the entire pressure range of the lower mantle. The pressure was determined on the basis of the Raman shift of diamond after the experiments and correction for the thermal pressure determined by the unit-cell volume of MgSiO_3 -rich perovskite at beamline **BL10XU**. The temperature, which was measured using a spectroradiometric method with a spatial resolution of $1\text{ }\mu\text{m}$, was quenched to room temperature by turning off the heating laser once the temperature reached the targeted value. The highest temperature with the $1\text{-}\mu\text{m}$ spatial resolution during heating was adopted as the experimental temperature in each run because melting occurs once the sample exceeds the solidus temperature. The temperature spatial resolution is higher than the size of the observed melt pocket by subsequent X-ray microtomographic imaging.

We also collected the X-ray microtomographic images of the recovered samples to explore the three-dimensional (3D) internal structure of all samples at beamline **BL47XU**. Some of the samples exhibit a round shaped Fe-enriched pocket at the center, which corresponds to the hottest part during laser heating (Fig. 2). Subsequent electron microprobe analyses confirmed that consistent with earlier melting experiment [2], such iron-rich regions represent quenched partial melts with nonstoichiometric compositions. Due to its high spatial resolution, the present microtomography imaging detected quenched melt pockets about $3\text{ }\mu\text{m}$ in size, which are formed by $\sim 3\text{ vol}\%$ partial melting at 142 GPa and 3690 K, but not at 151 GPa and 3680 K (Fig. 2). Thus, the solidus curve of pyrolite is bracketed as low as 3570(200) K at the CMB [3]. The solidus curve obtained in this study is consistent with the results of

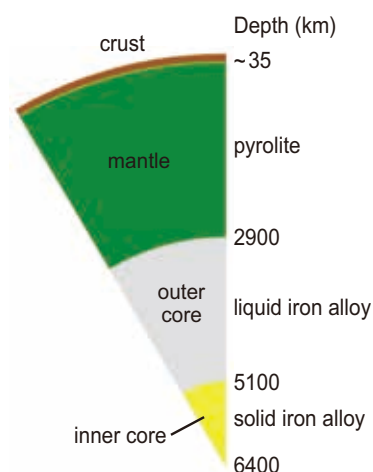


Fig. 1. Schematic illustration of the Earth's interior.

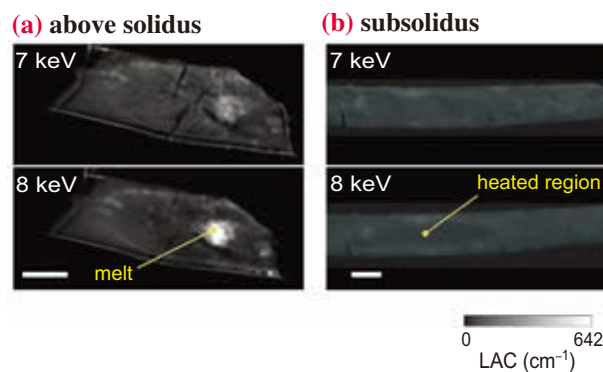


Fig. 2. Computed tomography images of a pyrolitic material quenched from the deep mantle conditions [4]. Brightness contrast is based on the X-ray linear attenuation coefficient (LAC) of an object. Comparison between the images obtained at 7 and 8 keV energies shows an Fe-rich melt pocket at the hottest part of the sample in A (142 GPa/3690 K) but not in B (151 GPa/3680 K). Scale bars represent 5 μm .

multi-anvil experiments below 25 GPa but is lower than those of recent laser-heated DAC studies combined with *in situ* X-ray diffraction measurements [1].

A lower solidus temperature helps explain the present day thermal and chemical structure of the mantle and the core. Although seismic observations may suggest the presence of a partial melt above the CMB, the possible partial melting is local feature not global one. Because the CMB temperature is isothermal, such a local occurrence indicates that these regions have distinct chemical compositions with lower melting temperatures. The solidus temperature of the mantle directly above the core is constrained by

the upper limit on the CMB temperature to 3570 (200) K, which is much lower than the conventional estimate of around 4000 K. Pure iron cannot melt at such low temperatures. The outer core is known to contain about 10 wt% light elements in addition to iron and nickel. An alloy with the appropriate amount of sulfur, silicon, and oxygen, which are the most plausible candidates for the light elements from cosmochemical and geochemical aspect of views, cannot melt the outer core. However, the 0.6 weight% (25 atomic%) hydrogen (Fig. 3) may have been incorporated into the core from the hydrous magma ocean, which is thought to have existed on Earth during core formation [5].

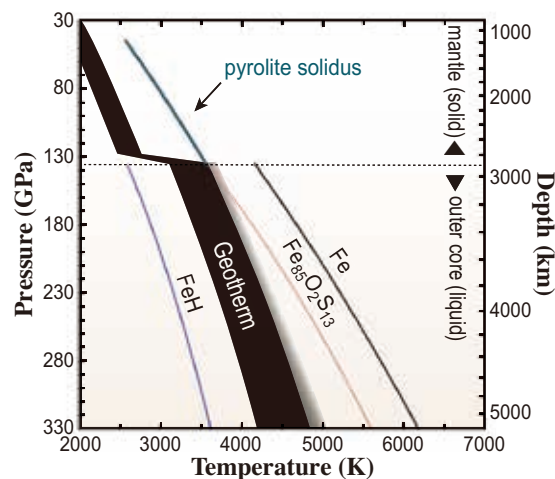


Fig. 3. Temperature profile (geotherm) in the lower mantle and the outer core [4]. Dark green curve depicts the solidus of pyrolite determined in this study. Melting (liquidus) temperatures of pure iron, Fe-O-S alloy, and FeH are shown by black, pink, and blue lines, respectively.

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References

- [1] G. Fiquet *et al.*: Science **329** (2010) 1516.
- [2] R. Nomura *et al.*: Nature **473** (2011) 199.
- [3] A. Tsuchiyama *et al.*: Geochim. Cosmochim. Acta **116** (2013) 5.
- [4] R. Nomura, K. Hirose, K. Uesugi, Y. Ohishi, A. Tsuchiyama, A. Miyake and Y. Ueno: Science **343** (2014) 522.
- [5] T. Okuchi: Science **278** (1997) 1781.

High-pressure radiative conductivity of dense silicate glasses and implications for dark-magmas at the Earth's core-mantle boundary

The current structure of the Earth's interior is believed to have formed through dynamic differentiation from a global magma ocean in the early Earth. Elucidation of the heat transport properties of silicate melts in the deep Earth is fundamental to understanding the evolution and structure of Earth's interior. The possible presence of dense, gravitationally stable silicate melts at the bottom of the current mantle as remnants of the deep magma ocean has been proposed to explain the anomalously low seismic velocities above the core-mantle boundary (e.g., Ref. 1). If this is the case, heat fluxes through the core-mantle boundary (CMB) region would strongly depend on the thermal conductivity due to both lattice vibrations (k_{lat}) and radiation (k_{rad}) of such dense silicate melts and the constituent minerals of the lower mantle. However, the thermal properties of such silicate melts under the relevant high-pressure conditions are poorly understood, even though there have been several experimental studies on the thermal conductivity of lower mantle minerals such as magnesium-rich silicate perovskite [2] and ferropericlase [3]. Direct measurements of the thermal conductivity in silicate melts under ultrahigh-pressure conditions remain a great challenge and are currently beyond experimental capabilities. As an alternative, silicate glasses have been studied as analogs for quenched silicate melts to simulate their high-pressure behavior. We conducted visible and near-infrared optical absorption and synchrotron Mössbauer spectroscopic measurements of iron-enriched silicate glasses at high pressures up to 85 GPa using a diamond anvil cell high-pressure apparatus to clarify the potential influences of the pressure-induced electronic structure changes in dense silicate melts on the radiative part of the thermal conductivity [4].

Two types of silicate glasses were used as analogs for dense silicate melts at the CMB: $(\text{Mg}_{0.8}\text{Fe}_{0.2})\text{SiO}_3$ composition (E-glass) to extract the effect of iron and a multicomponent basaltic composition (M-glass) to simulate a more realistic compositional system at the CMB. In the optical absorption measurements, we observed significant increases in the absorption coefficients with increasing pressure (up to a factor of three for E-glass, and an order of magnitude for M-glass), particularly in the visible region above wavenumbers of $\sim 10,000\text{ cm}^{-1}$, and both samples apparently become optically darker with pressure, as shown in Fig. 1.

In situ high-pressure synchrotron Mössbauer spectroscopic measurements in the energy domain

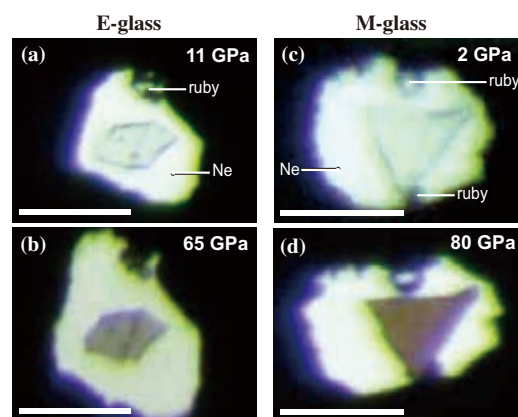


Fig. 1. Optical images of the glass samples under pressure in a diamond anvil cell. (a) E-glass at 11 GPa and 300 K, (b) E-glass at 65 GPa and 300 K, (c) M-glass at 2 GPa and 300 K, and (d) M-glass at 80 GPa and 300 K. Pressure medium is neon and the white scale bar is 100- μm long.

were performed on E-glass using beamline BL11XU [5]. As shown in Fig. 2(a), statistically sufficient Mössbauer spectra were obtained using a measurement time between 3 and 6 hours (Fig. 2(a)). Hyperfine structure parameters were determined by distribution analysis of the Mössbauer spectra (Fig. 2(b,c)). Above 20 GPa, a new quadrupole doublet (Sites 1 and 2) appears, which is derived from a low isomer shift (IS, $\sim 0.67\text{ mm/s}$ relative to $\alpha\text{-Fe}$) and moderate quadrupole splitting (QS, $\sim 1.6\text{ mm/s}$). If the nearest neighbor atoms around iron are oxygen, in principle, the IS should increase with an increase of the Fe-O coordination number. However, if the IS is derived from the new quadrupole doublet, it should show the opposite trend (Fig. 2(b)). Alternatively, this trend can be interpreted as a change in the electronic state in the absence of significant changes in the coordination number, which may be related to the formation of Fe^{2+} with a different electronic configuration from that expected at lower pressures. Given that the IS for Fe^{2+} with low spin states in common compounds is around -0.3 to 0.4 mm/s , our IS data, which has intermediate values between high and low spin, indicates the appearance of Fe^{2+} with an intermediate spin state, rather than a complete transition to low spin states.

The present data obtained from silicate glasses with representative compositions for a dense silicate melt suggests the possible effect of pressure-induced changes in the electronic configurations, including the spin states of iron, on the thermal properties of the melt. The radiative component of the thermal conductivity, $k_{\text{rad}}(T)$, can be then calculated as:

$$k_{rad}(T) = \frac{4n^2}{3} \int_0^\infty \frac{1}{\alpha} \frac{\partial I(\nu, T)}{\partial T} d\nu \quad (1)$$

where n is the refractive index, α is the measured absorption coefficient, $I(\nu, T)$ is the Planck function, and T is temperature. The radiative conductivity increases with temperature but decreases with pressure for both components, as shown in Fig. 3. The radiative thermal conductivity of materials with a constant absorbance was believed to be proportional to the third power of temperature (i.e., T^3). However, the temperature dependence of the radiative thermal conductivity estimated in the present study is significantly lower than that expected for the T^3 relationship. The radiative conductivities for both components increase only by a factor of 8–9 from 2000 K to 7000 K, which is about five times lower than expected for a T^3 dependence.

The significantly smaller contribution to heat transfer by radiative conductivity of a silicate melt strongly affects the heat flow at the CMB. The results presented here indicate that the estimated radiative thermal conductivity of a basaltic melt, calculated from the results of M-glass, under the lowermost mantle conditions (at ~ 130 GPa and ~ 3000 K), is ~ 0.19 $\text{m}^{-1}\text{K}^{-1}$. However, dense silicate melts with basaltic compositions are about 5 to 25 times less radiatively conductive than the silicate perovskite phase with representative iron contents in the lower mantle under conditions at the base of the mantle. This remarkable contrast at high pressures indicates the formation of deep magmas with higher heat absorption than that of the surrounding solid mantle phases. Such dense and dark magmas at the CMB may act as traps for heat from the underlying outer core. Seismological

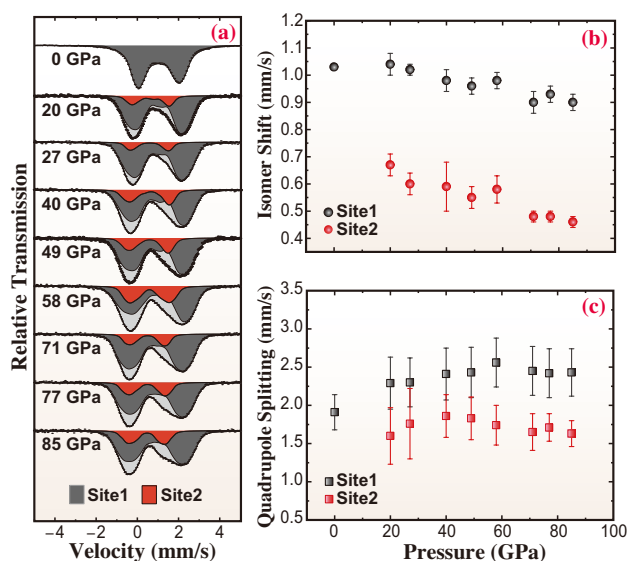


Fig. 2. Mössbauer transmission spectra determined for E-glass between (a) 0 and 85 GPa and (b, c) hyperfine structure parameters. (b) Isomer shift and (c) quadrupole splitting. Error bars are $\pm 1\sigma$ from the mean value.

mapping at the CMB by detailed waveform modeling studies shows that the ultralow velocity zones (ULVZs) exist as laterally distributed thin layers over a wide region. The distribution of ULVZs correlates strongly with flux-weighted hot spot locations, which have been interpreted as a large-scale lower mantle thermal upwelling that is referred to as a “superplume.” The presence of a heterogeneous distribution of such dense magmas with lower radiative thermal conductivity would result in a lateral heterogeneity of heat fluxes through the CMB, which may constrain the locations of stable hot mantle plumes rooted in CMB.

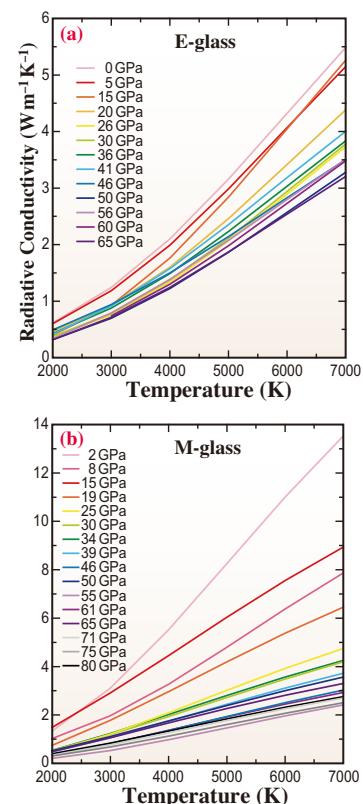


Fig. 3. Estimated radiative thermal conductivity of (a) E-glass and (b) M-glass as functions of temperature up to 7000 K.

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References

- [1] E. Ohtani & M. Maeda: *Earth Planet. Sci. Lett.* **193** (2001) 69.
- [2] A.F. Goncharov *et al.*: *Nature* **456** (2008) 231.
- [3] A.F. Goncharov *et al.*: *Science* **312** (2006) 1205.
- [4] M. Murakami, A.F. Goncharov, N. Hirao, R. Masuda, T. Mitsui, S.-M. Thomas, C.R. Bina: *Nat. Commun.* **5** (2014) 5428.
- [5] T. Mitsui *et al.*: *J. Synchrotron Rad.* **16** (2009) 723.

Elastic anisotropy of experimental analogs of perovskite and post-perovskite by means of inelastic X-ray scattering

Recent studies have shown that the D" layer, just above the Earth's core-mantle boundary, is composed of MgSiO_3 post-perovskite and has a significant lateral inhomogeneity. Here we consider the D" diversity as related to the single crystal elasticity of the post-perovskite phase. We measured the single-crystal elasticity of the perovskite $Pbnm\text{-CaIrO}_3$ and post-perovskite $Cmcm\text{-CaIrO}_3$ using inelastic X-ray scattering (IXS) at beamline **BL35XU** [1]. These materials are structural analogs to same phases of MgSiO_3 . Our results show that $Cmcm\text{-CaIrO}_3$ is much more elastically anisotropic than $Pbnm\text{-CaIrO}_3$, which gives an explanation for enigmatic seismic wave velocity jump at the D" discontinuity. Considering a relation between lattice preferred orientation and seismic anisotropy in the D" layer, we suggest that the c -axis of post-perovskite MgSiO_3 aligns vertically beneath the Circum-Pacific Rim, and the b -axis vertically aligns beneath the Central Pacific.

Since the discovery of the transition from perovskite (Pv) to post-perovskite (pPv) in MgSiO_3 [2], pPv- MgSiO_3 has been considered to be a major component of the D" layer just above the core-mantle boundary (CMB). Seismological observations of the D" layer are difficult to interpret as they suggest both non-uniform response at the layer boundary and non-uniform anisotropy inside the layer [3]. With this background, elastic anisotropy, or single-crystal elasticity, of pPv- MgSiO_3 has been the focus of theoretical calculations [4]. However, no experimental data are available on the single-crystal elasticity of pPv- MgSiO_3 , because it is unstable at ambient pressure.

$Cmcm\text{-CaIrO}_3$, or pPv- CaIrO_3 , has been frequently selected as a representative analog of pPv- MgSiO_3 . According to the phase diagram, pPv- CaIrO_3 is the stable phase at ambient conditions and Pv- CaIrO_3 is the high-temperature and low-pressure phase. Here we report the first experimental data on single crystal elasticity of Pv and pPv structures in a homogeneous chemical composition of CaIrO_3 .

Figures 1(A) and 1(B) show single crystals of Pv- CaIrO_3 and pPv- CaIrO_3 used in the present study. The Pv- CaIrO_3 sample was synthesized at 2 GPa and 1450°C for 15 h in a piston cylinder apparatus at Okayama University. We produced many grains on the order of a few tens of microns, but most of them turned out to have multiple domains. We examined more than 20 grains using a four-circle X-ray diffractometer at Yamaguchi University and selected a quasi-single domain crystal with a size of $\sim 20\ \mu\text{m}$.

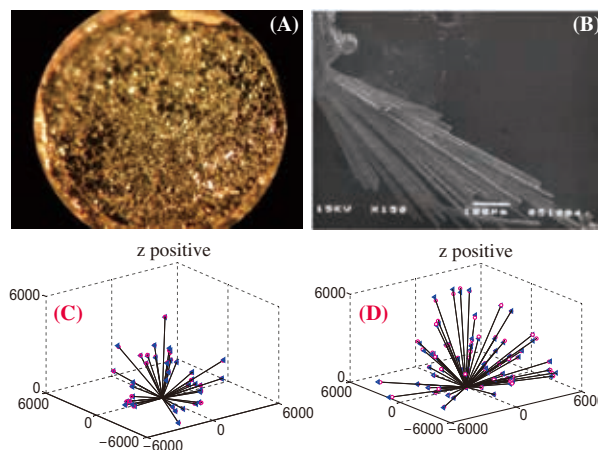


Fig. 1. Specimen and measurement data. Left and right columns refer to $Pbnm\text{-CaIrO}_3$ and $Cmcm\text{-CaIrO}_3$, respectively. (A, B) Photographs of the single crystals used. Horizontal edge lengths are $\sim 5\ \text{mm}$ and $\sim 800\ \text{mm}$ for A and B, respectively. (C, D) Overall picture for the experimental data and analytical fitting. Black lines indicate the direction of wave propagation in the crystal. Blue triangles and red circles are experimental velocities and recalculated values after the least-squares analysis, respectively. Vertical direction is the positive z direction, while horizontal leftward and rightward directions are positive x and y directions, respectively. Note that the difference between experimental and recalculated velocities is less than 5%. Uniformly distributed misfit indicates untwined crystal specimens.

Its lattice constants were determined as $a = 5.3527(1)$, $b = 5.5969(5)$, and $c = 7.6804(6)\ \text{\AA}$, which yields a density (ρ) of $8091\ \text{kg/m}^3$. The pPv- CaIrO_3 sample was synthesized by slowly cooling from 1000°C in CaCl_2 flux. Figures 1(C) and 1(D) compare the experimental velocities obtained from the IXS spectra with velocities calculated from the optimized elastic constants.

According to seismological studies, the region of the Circum-Pacific Rim is interpreted having a vertically transverse isotropy (VTI) with $V_{SH} > V_{SV}$, while the region of the Central Pacific has a complicated VTI with many relations between V_{SH} and V_{SV} [3]. Either polarization anisotropy is not recognized beneath the Atlantic Ocean, or the region is considered as having VTI with $V_{SH} \approx V_{SV}$. On the other hand, from the polarization pattern in the a - b plane of the faster V_S plot (Fig. 2(B)), we can expect that $V_{SH} > V_{SV}$ in the D" layer if the c -axis of pPv is aligned vertically. Similarly, if the b -axis aligns vertically, the magnitude relation between V_{SH} and V_{SV} can be complicated.

We can summarize the above conclusion in alternative way: for the c -axis vertical case, the horizontal wave propagation directions lie in the a - b plane, which the in-plane shear mode (SH) has consistently higher wave speeds (Fig. 2(B)). However,

if instead the b -axis is vertical, the horizontal wave propagation directions lie in the a - c plane, which neither the in-plane (SH) nor the out-of-plane (SV) is consistently faster for all such propagation directions (Fig. 2(B)).

The dominant slip system of pPv-CalrO₃ was determined as [100](010) from the shear deformation experiments. Since the slip system of pPv-MgSiO₃ has not yet been determined, we assumed that it is similar to that of pPv-CalrO₃. This assumption leads to LPO of pPv-MgSiO₃ in which the b -axis aligns vertically along the lateral flow in the D" layer. The LPO pattern is consistent with the complicated polarization S-wave anisotropy beneath the Central Pacific (Fig. 2(B)).

Although the textural development during Pv-pPv transition in MgSiO₃ has not yet been clarified, we expect the c -axis alignment of pPv-MgSiO₃ immediately after the phase transition from Pv-MgSiO₃ from analogy with the case for MgGeO₃. If the c -axis aligns vertically, we expect VTI with $V_{SH} > V_{SV}$, which is consistent with the seismic feature beneath the Circum-Pacific Rim.

Figure 3 summarizes the present idea for the seismic anisotropy and LPO structure in the D" layer. The VTI of the vertical c -axis and that of the vertical b -axis can be interpreted as transformation LPO and deformation LPO, respectively. It is noted that isotropic pPv-MgSiO₃ is a reasonable interpretation for the seismic feature beneath the Atlantic Ocean; an alternative interpretation may be the VTI of the vertical a -axis because of less lateral polarization anisotropy than in other cases. The difference between beneath the

Pacific and Atlantic may be attributed to differences in temperature, chemical composition, and strain rate. The proposed LPO behavior for pPv-MgSiO₃ obtained from the present mineral physics perspective is consistent with seismic observation.

In this study, we presented a new data set of Pv and pPv-CalrO₃ single-crystal elasticity by means of IXS at BL35XU. We used the present results to interpret the seismic wave anisotropy in the D" layer and proposed a model of pPv LPO in the D" layer consistent with seismic observations and the mineral physics experiments on analog materials. The present model may allow a sophisticated discussion about global mantle convection, which is triggered by heating at the CMB and modulated by lateral flow in the D" layer.

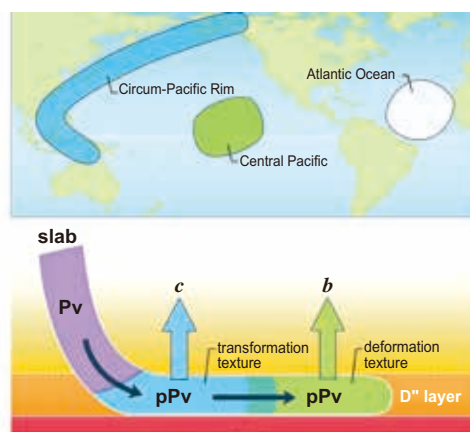


Fig. 3. Schematic illustration of the LPO of pPv-MgSiO₃ in the D" layer. From a mineral physics viewpoint, the Pv slab (pink region) transforms into pPv (light blue region) and then the pPv slab moves along the path of mantle convection with deformation (light-green region). In contrast, from seismic observations, the region of the Circum-Pacific Rim has VTI anisotropy of $V_{SH} > V_{SV}$, while the region of the Central Pacific has complicated VTI with an undetermined magnitude relation, i.e., $V_{SH} > V_{SV}$, $V_{SH} < V_{SV}$ or $V_{SH} \approx V_{SV}$. Region of the Atlantic Ocean (white circle) has VTI with $V_{SH} \approx V_{SV}$, suggesting isotropy or negligible LPO of pPv MgSiO₃. The speculated aligned axes of pPv LPO are shown by arrows for each region; the vertical c -axis is beneath the Circum Pacific Rim and the vertical b -axis is beneath the Central Pacific.

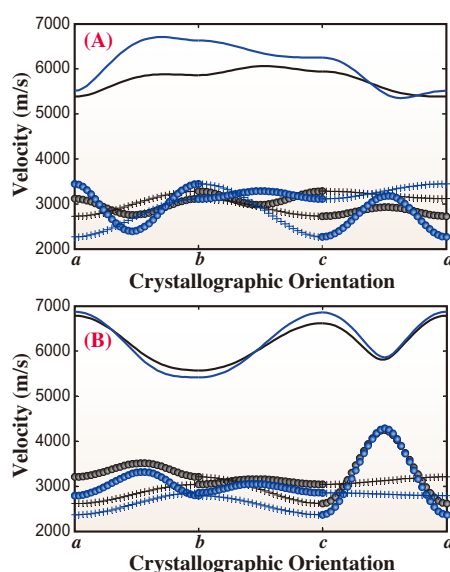


Fig. 2. Velocity surface of $Pbnm$ - and Cmc -CalrO₃. (A) Upper and (B) lower plots correspond to $Pbnm$ - and Cmc -CalrO₃, respectively. Black lines are drawn for the present results, while blue lines are drawn for the results obtained in Ref. 5. Upper lines correspond to the P-wave velocity, and lower lines to the S-wave velocity. Solid circles and crosses correspond to V_s specify in-plane and out-of-plane particle motions.

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References

- [1] A. Yoneda, H. Fukui, F. Xu, A. Nakatsuka, A. Yoshiasa, Y. Seto, K. Ono, S. Tsutsui, H. Uchiyama and A.Q.R. Baron: *Nat. Commun.* **5** (2014) 3453.
- [2] M. Murakami *et al.*: *Science* **304** (2004) 855.
- [3] A. Nowacki *et al.*: *J. Geodyn.* **52** (2011) 205.
- [4] R.M. Wentcovitch & J. Tsuchiya: *PNAS* **103** (2006) 543.
- [5] T. Tsuchiya & J. Tsuchiya: *Phys. Rev. B* **76** (2007) 144119.

Transportation of water into the deep lower mantle by new type of dense hydrous magnesium silicate

Water is a key component in the Earth's evolution and dynamics because it strongly influences many chemical and physical properties of the mantle materials. In a descending slab, a certain amount of water is transported into the Earth's interior via dense hydrous magnesium silicate (DHMS). Phase D, which is one form of DHMS, is known to have large stability fields and be stable in the upper part of the lower mantle conditions. According to the previous experimental study, the transportation of water by DHMS would be terminated in the middle of the lower mantle (~1250 km depth) due to the dissociation of phase D into assemblages of MgSiO_3 bridgmanite plus water. On the other hand, a new type of DHMS has been theoretically predicted to be stable at pressures above ~45 GPa [1]. A multi-anvil apparatus using sintered diamond anvils in conjunction with a synchrotron X-ray diffraction technique allows this theoretical prediction to be confirmed. We are the first to experimentally show that phase D transforms to an assemblage with a new DHMS, named phase H, at pressures above ~48 GPa [2].

In situ X-ray diffraction measurements were performed using a multi-anvil apparatus (SPEED-Mk.II) at beamline BL04B1. White X-rays from synchrotron radiation were used as the incident beam. Sintered diamond anvils with a truncated

edge length of 1.5 mm were used as the second-stage anvils to generate pressures up to 50 GPa. The chemical compositions of the starting materials were MgSiO_4H_2 and $\text{MgSi}_{1.5}\text{H}_2\text{O}_5$, which were prepared by mixing $\text{Mg}(\text{OH})_2$ brucite and SiO_2 silica. The diffracted beam was measured using the energy-dispersive method with a fixed angle of 6.0° and a Ge solid-stage detector. A small amount of Au powder mixed into the samples was used to determine the pressure.

At 400°C , 37.3 GPa and a starting composition of MgSiO_4H_2 , broad diffraction peaks of $\text{Mg}(\text{OH})_2$ brucite and the Au pressure marker were observed (Fig. 1(a)). At 900°C and 37.3 GPa, peaks corresponding to phase H appeared and remained at least up to 42.0 GPa and $1,000^\circ\text{C}$ (Fig. 1(b)). BSE images of the recovered sample show an almost single phase of phase H (Fig. 2(a)). Additional quenching experiments indicated the formation of phase H at least up to 50 GPa and 1000°C . Using a starting composition of $\text{MgSi}_{1.5}\text{H}_2\text{O}_5$, phase D transformed to phase H plus stishovite around 48 GPa (Fig. 2(b)). These reactions and transition boundaries are consistent with those predicted by the theoretical calculation [1]. The diffraction peaks of phase H were indexed with an orthorhombic symmetry identical to a high-pressure hydrous phase $\delta\text{-AlOOH}$, yielding $a = 4.478 \text{ \AA}$, $b = 4.091 \text{ \AA}$ and $c = 2.700 \text{ \AA}$ at 42 GPa and $1,000^\circ\text{C}$. This structure slightly differs from the

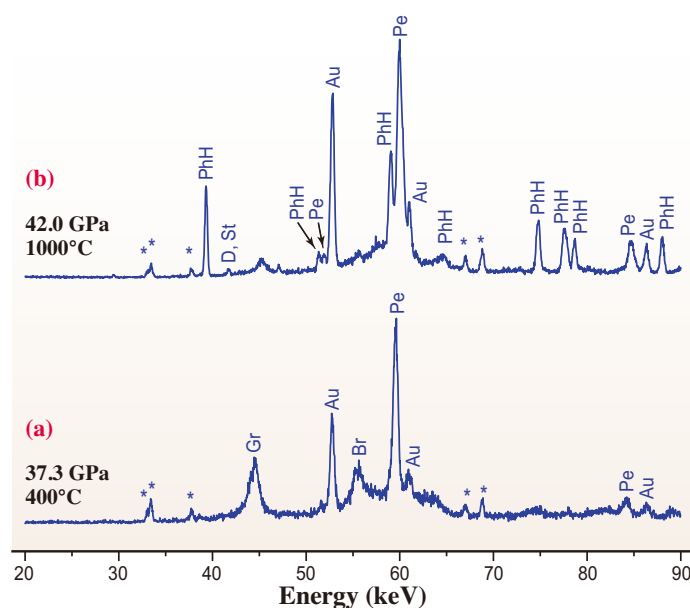


Fig. 1. X-ray diffraction patterns of *in situ* observations at BL04B1. PhH, phase H; St, stishovite; Br, brucite; Pe, periclase; Gr, graphite; D, phase D; *, characteristic lines of Au (gold) and La (lanthanum).

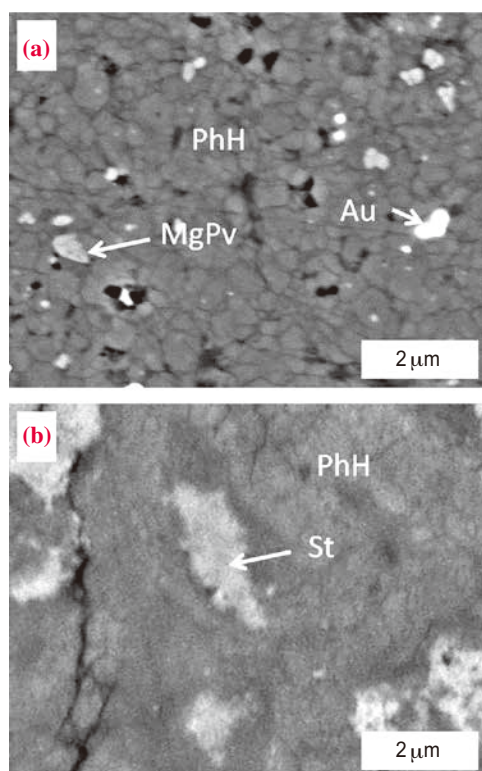


Fig. 2. Back-scattered electron images of the run products. (a) 42 GPa and 1000°C with the composition of MgSiH_2O_4 phase H (PhH). (b) 50 GPa and 950°C with the composition of $\text{MgSi}_{1.5}\text{H}_2\text{O}_5$ phase D. St: stishovite, MgPv: bridgmanite, Br: brucite, Au: gold.

theoretical prediction, which possesses a monoclinic symmetry. On the other hand, white X-ray diffraction measurements did not provide detailed structural data at ambient conditions due to the rapid amorphization of the sample during observation. Therefore, we also tried to investigate the structure of phase H at ambient conditions by single-crystal X-ray diffraction; the structure is orthorhombic in space group $Pn\bar{n}m$ (CaCl_2 -type structure, Fig. 3) with lattice parameters $a = 4.733$, $b = 4.3250$, $c = 2.8420$ Å, $V = 58.18$ Å³ [3].

In additional experiments, we confirmed that phase H forms a solid solution with $\delta\text{-AlOOH}$ by substitution of $\text{Mg}^{2+} + \text{Si}^{4+} \rightleftharpoons \text{Al}^{3+}$ because of their similar crystal structures. Moreover, alumina is strongly partitioned into phase H relative to coexisting MgSiO_3 bridgmanite. Our experimental results and the theoretical calculation indicate that the stability field of end-member MgSiO_4H_2 phase H is limited up to ~55 GPa and 1000°C, which correspond to the conditions of the middle lower mantle in a cold subducting plate. On the other hand, it is known that $\delta\text{-AlOOH}$ has an extremely large stability field over the entire range of conditions in the lower mantle [4]. Therefore, the stability field of phase H in the actual mantle should be

expanded towards a higher pressure and temperature since aluminium is a common element in Earth.

Recent experimental results using a diamond anvil cell (DAC) at beamline **BL10XU** also support a largely stable phase H. Phase H - $\delta\text{-AlOOH}$ solid solution (aluminous phase H) is stable up to the conditions of the base in the lower mantle and coexists with MgSiO_3 bridgmanite and post-perovskite [5]. Thus, aluminous phase H may be stable at pressures and temperatures corresponding to the conditions of whole lower mantle and should be capable of delivering a certain amount of water into the deepest regions of the lower mantle.

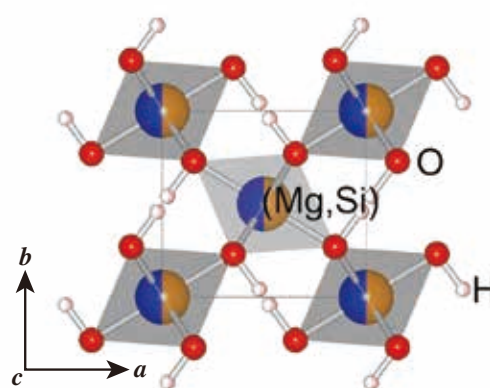


Fig. 3. Crystal structure of MgSiH_2O_4 phase H determined by the single crystal X-ray diffraction method [3]. Large bi-colored spheres indicate the atomic positions where Mg and Si atoms are disordered. Medium spheres are oxygen atoms. Small bi-colored spheres indicate hydrogen atoms with half-occupations to avoid forming adjacent H-H bonding.

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References

- [1] J. Tsuchiya: *Geophys. Res. Lett.* **40** (2013) 4570.
- [2] M. Nishi, T. Irifune, J. Tsuchiya, Y. Tange, Y. Nishihara, K. Fujino, Y. Higo: *Nat. Geosci.* **7** (2014) 224.
- [3] L. Bindi *et al.*: *Am. Mineral.* **99** (2014) 1802.
- [4] A. Sano *et al.*: *Phys. Earth Planet. Inter.* **35** (2008) L03303.
- [5] I. Ohira *et al.*: *Earth Planet. Sci. Lett.* **401** (2014) 12.

Detection of uranium and chemical state analysis of individual radioactive microparticles emitted from the Fukushima nuclear accident using multiple synchrotron radiation X-ray analyses

The Fukushima Daiichi Nuclear Power Plant (FDNPP) accident was the most catastrophic nuclear incident since the 1986 Chernobyl disaster, and has been rated at the maximum level of 7 on the International Nuclear Event Scale. Large amounts of radioactive materials were released into the environment during the accident [1,2]. Although more than three years have passed, radioactive materials emitted from the FDNPP can still be detected in the environment. However, little is known about the physical and chemical natures of radioactive materials released during the early stages of the accident. Spherical microparticles ($\sim 2 \mu\text{m}$ ϕ) containing radioactive Cs were found in aerosol samples collected on 14 and 15 March 2011 in Tsukuba, which is 172 km southwest of the FDNPP using a scanning electron microscope (SEM) with an energy dispersive X-ray spectrometer (EDS) [3]. These microparticles consisted of Fe, Zn, and Cs and were insoluble in water.

We have conducted a more detailed study of the nature of the Cs-bearing radioactive aerosol microparticles using advanced analytical techniques and a synchrotron radiation (SR)-X-ray microbeam at beamline BL37XU [4]. X-ray fluorescence (XRF) analysis using a high-energy SR-X-ray microbeam, which is much more sensitive to heavy elements than SEM-EDS analysis, was used to carry out nondestructive identification and qualitative detection of trace amounts of heavy elements in individual microparticles. To evaluate the conditions under which these particles were formed, chemical state analysis of the transition elements in the particles was performed by applying X-ray absorption near-

edge structure (XANES) analysis and to reveal the crystal structures of the particles, X-ray diffraction (XRD) analysis was conducted. Brilliant X-rays from an advanced SR light source at SPring-8 enabled us to use a combination of these three analytical techniques. We applied these analytical techniques to the Cs-bearing microparticles from the FDNPP accident to reveal their nature and to further understand the accident as well as the effects on the environment and human health. Three radioactive particles, designated as particles A, B, and C, were sampled from the aerosol filter and placed on a flat Kapton® tape with a plastic holder for SR X-ray analyses. All samples were spherical with diameters of $\sim 2 \mu\text{m}$. Here we report the analytical results of particle A as an example.

The SR- μ -XRF spectra of particle A and the carbon tape background are shown in Fig. 1. In addition to Fe, Zn, and Cs, which were previously reported [3], the following 11 heavy elements were also detected in the particle: Cr, Mn, Rb, Zr, Mo, Sn, Sb, Te, Ba, Pb, and U. Figure 2 shows the distribution of selected elements from the SR- μ -XRF imaging of particle A in a SEM image corresponding to the imaging area. In this particle, the two-dimensional distributions of characteristic elements, including U, correspond well to the particle shape in the SEM image and the Cs distributions identified by the SEM-EDS analysis. To obtain additional evidence for the presence of U in the microparticle, we conducted U- L_3 edge SR- μ -XANES analysis on particle A (Fig. 3(a)). A clear edge jump is observed at the energy of the U- L_3 edge, confirming the presence of U within the

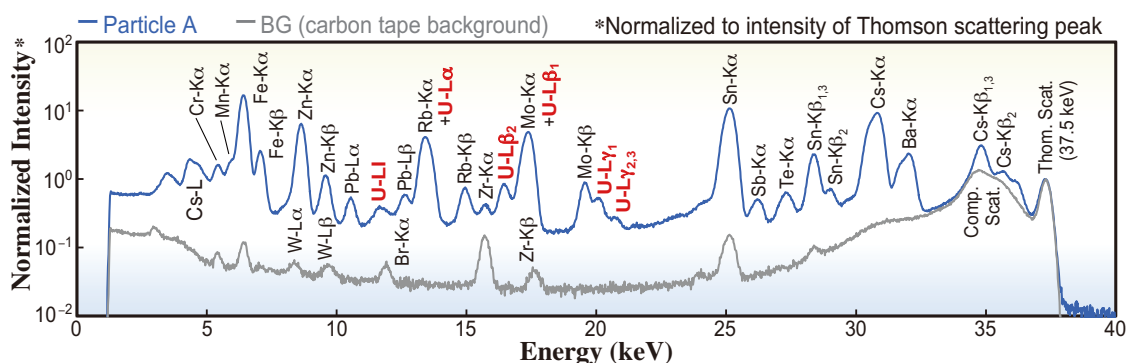


Fig. 1. Comparison of the SR- μ -XRF spectra obtained for particle A and the carbon tape background. Intensity of each spectrum is on a logarithmic scale.

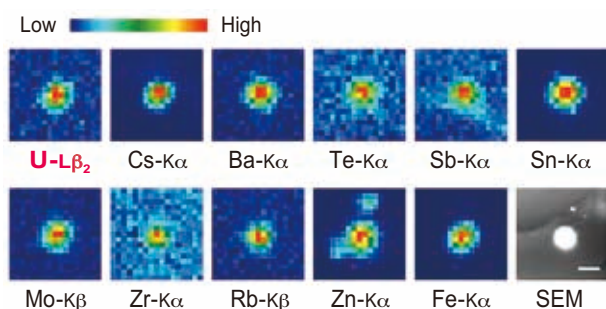


Fig. 2. Distributions of representative elements extracted from the SR- μ -XRF images of particle A with an enlarged SEM image corresponding to the imaging area (scale bar: 2 μ m).

particle in the environment. This result implies that in addition to Cs from reactor, elements related with nuclear fuel were also emitted into the atmosphere.

The SR- μ -XANES spectra of the Fe, Mo and Sn for the particle are shown in Fig. 3(b-d), respectively. The peak positions and the shapes of the pre-edges for particle A and the reference materials are consistent, indicating that these elements occur as Fe^{3+} , Mo^{6+} , and Sn^{4+} in the glass matrix. In addition, SR- μ -XRD pattern of particle A shows that the particle does not exhibit a diffraction peak, suggesting that the particle is an amorphous, glassy material. These characteristics imply that the microparticle may have a relatively long-term impact on the environment, i.e., continued release of soluble radioactive Cs into the environment as these insoluble glassy particles degrade.

We then explored the possible sources of the 13 elements (Cr, Mn, Fe, Zn, Rb, Zr, Mo, Sn, Sb, Te, Cs, Ba, and U) found within the microparticles by SR- μ -XRF analysis. The nuclear fission reaction of U may produce 8 of the elements (Rb, Zr, Mo, Sn, Sb, Te, Cs, and Ba) as fission products (FPs). A Zr-Sn alloy was used for fuel cladding within the reactors. Stainless steel, which commonly consists of Fe, Cr, and Mn, was used in the vessel structure. Zn was added to the primary cooling water in the FDNPP for corrosion control to reduce ^{60}Co . Therefore, we conclude that the U fuel, FPs, and components of the reactors are very likely the sources of the elements identified within the radioactive microparticle. Because these elements likely originated from multiple sources, we assume that they melted together during the accident and eventually formed spherical microparticles.

Clarifying the nature of these microparticles should assist in understanding what occurred in the reactors during the early stages of the accident. Accurate simulations of the distribution and deposition of radioactive materials depend on the physical and chemical natures of the materials of interest. Our results should help improve models simulating how the radioactive materials were formed and distributed from the reactors into the environment during the accident. Further quantitative investigations of the chemical nature of the radioactive particles, including quantification and chemical state analysis of U and FPs, will be important to further understand the mechanisms of particle formation and emissions as well as their potential human health and environmental impacts.

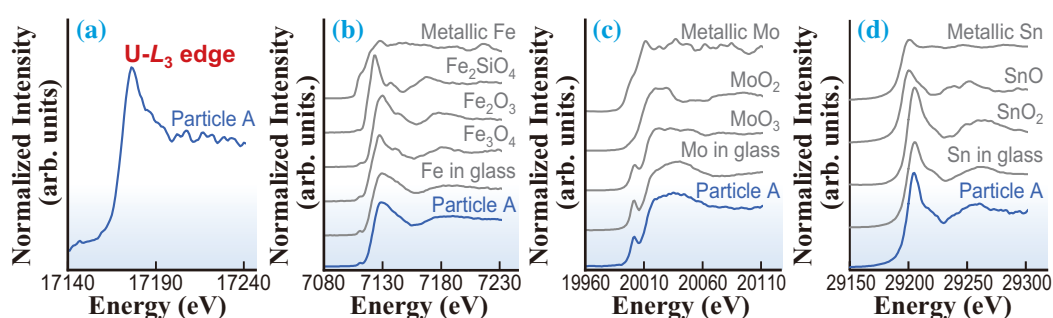


Fig. 3. Results of SR- μ -XANES analyses. (a) U- L_3 edge SR- μ -XANES spectrum of particle A, demonstrating the presence of U in the particle. Comparisons of the (b) Fe- K edge, (c) Mo- K edge, and (d) Sn- K edge SR- μ -XANES spectra of particle A and the reference materials.

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References

- [1] MEXT, Japanese Ministry of Education, Culture, Sports, Science and Technology. <http://www.mext.go.jp/english>, accessed on 14th January 2015.
- [2] N. Yoshida, J. Kanda: Science **336** (2012) 1115.
- [3] K. Adachi *et al.*: Sci. Rep. **3** (2013) 2554.
- [4] Y. Abe, Y. Iizawa, Y. Terada, K. Adachi, Y. Igarashi, I. Nakai: Anal. Chem. **86** (2014) 8521.

The chemical form of sulfur compounds in the Japanese pink coral (*Corallium elatius*) skeleton using μ -XRF/XAS speciation mapping

As established in the pioneering work by Smith *et al.* [1], trace elements in biogenic calcium carbonates are well known as highly valuable records of paleoenvironmental changes since the annual growth band recorded in the hard skeleton is a time stamp of the past and the incorporation of trace elements is closely related with various environmental parameters such as temperature, pH, nutrients, and water salinity. The Mg/Ca and Sr/Ca ratios in biogenic calcium carbonates are good examples of elements with the potential to be used as proxies; these ratios have been successfully used as a seawater thermometer. On the other hand, thus far, the chemical form of trace elements in biogenic carbonates is rarely considered in paleoclimatological investigations, and uncertainties of their chemical form remain. Because an inaccurate understanding of the chemical environment of trace elements may result in an errant interpretation in climate reconstruction, speciation analysis in order to establish the chemical form of trace elements in biogenic carbonates is an active field in paleoclimatology.

X-ray analysis using energy-tunable synchrotron radiation should be an attractive tool for simultaneous elucidation of the chemical form and distribution of trace elements via speciation analysis. X-ray photoabsorption spectroscopy (XAS) is a nondestructive analytical technique to characterize and identify trace elements in compositionally complex natural materials. In particular, XAS analysis of sulfur is an established technique, which is sensitive to the electronic structure, valence state, and local symmetry of the absorbing site and is useful as a fingerprint of the chemical form of elements in materials. Furthermore, the combination of XAS and X-ray fluorescence analysis (XRF) using a micro-focused X-ray beam allows both the chemical state of environmentally important trace elements and their spatial distribution in biogenic carbonates to be determined. In the present study, the μ -XRF/XAS technique was applied to the skeleton of Japanese pink coral (*Corallium elatius*; Fig. 1), to elucidate the chemical form of sulfur and clarify the distribution of chemically distinguishable sulfuric compounds [2].

All experiments were performed at BL27SU [2]. Figure 2 shows the XAS spectra of *C. elatius* and reference materials measured at the sulfur *K*-edge. For both coenenchyme and the coral skeleton, the XAS spectra show a major peak at 2481.9 eV. CaSO_4 and protamine sulfate are typical standard materials for determining the inorganic and organic sulfate



Fig. 1. Morphology of the Japanese pink coral skeleton (*C. elatius*). (a) Overview of the coral skeleton. White arrow indicates the analyzed portion. (b) Cross section of the slab cut perpendicular to the growth axis. (c) Two copper rings (ϕ 1-mm ring for coenenchyme and ϕ 2-mm for the coral skeleton interior) denoting the areas in which the μ -XAS/XRF measurements are performed. Red circle indicates the area in which the speciation mapping analysis is performed (see Fig. 3).

(SO_4^{2-}) content, and the white line at 2481.9 eV is a characteristic fingerprint of sulfate. Therefore, sulfate is the primary species of sulfur in both the coral skeleton and coenenchyme. On the other hand, the XAS spectrum for coenenchyme shows an additional peak at 2472.7 eV (blue curve). Cysteine and methionine are standard organic sulfur materials containing H–S–R and R–S–R bonds, respectively, with their main peaks at 2472.7 eV. These molecules are typical biological molecules containing sulfur. The coenenchyme spectrum suggests the presence of organic sulfur.

Figure 3 presents μ -XRF/XAS speciation mapping of sulfur on the outer edge of the specimen. Figure 3(f) shows a microscope image of the specimen surface for which speciation mapping was performed. The indicated area corresponds to the area labeled with a small Cu ring (ϕ = 1 mm) (Fig. 1(c)). The red area on the right side of the micrograph is the coral skeleton, while the central white area is the coenenchyme. The transparent fragments observed in the coenenchyme are sclerites. The dark area on the left side of the micrograph is the epoxy resin.

The mapping images were obtained by fixing the excitation energy at typical energies for oxidized (2481.9 eV) and reduced sulfur (2472.7 eV). These excitation energies are assigned to the characteristic resonance peaks for sulfate (SO_4^{2-}) and organic sulfur with S–C and/or S–H bonds (Fig. 2). Therefore, these mapping images exhibit the distributions of sulfate and organic sulfur, respectively. The speciation mapping clearly demonstrates that the distribution of sulfur strongly depends on its chemical form and that the

distributions of sulfate and sulfur-containing organic molecules in the *C. elatius* skeleton differ substantially. Sulfate is identified as the major species in the coral skeleton, whereas organic sulfides are relatively limited (Fig. 3(a)). In contrast, both organic sulfur and sulfate have higher intensities in the coenenchyme (Fig. 3(b)). Furthermore, the distributions of organic sulfur and sulfate in the coenenchyme contrast (Fig. 3(c)), as indicated by the color-coded composite image for the two sulfur compounds. The red and blue areas are attributed to high concentrations of sulfate and organic sulfur, respectively. Figures 3(c) illustrates the sulfate-rich parts of the coral skeleton, embedded with areas of organic sulfur. Figures 3(d) and 3(e) show the elemental mapping images for Mg and P, respectively. The distribution of P is strongly correlated with the distribution of organic sulfur. Thus, organic sulfur is associated with the distribution of biological molecules. Furthermore, in the coenenchyme, the obtained μ -XRF/XAS image indicates that the chemical form of sulfur depends on the biological tissue. Sulfate-rich areas of the coenenchyme are assigned to sclerite, which is composed of a fragmented calcite skeleton distributed in the soft tissues. The distribution of Mg also coincides with that of sulfate in the coenenchyme. Because the major component of sclerite is calcium carbonate, the distribution of Mg in the coenenchyme can be interpreted as substitution for Ca.

A μ -XRF/XAS mapping analyses clarifies that the spatial distributions of sulfur compounds in Japanese pink coral (*C. elatius*) is strongly dependent on the chemical form. Inorganic sulfate species (SO_4^{2-}) are distributed in both the coenenchyme and coral skeleton and the distribution pattern shows dark and bright

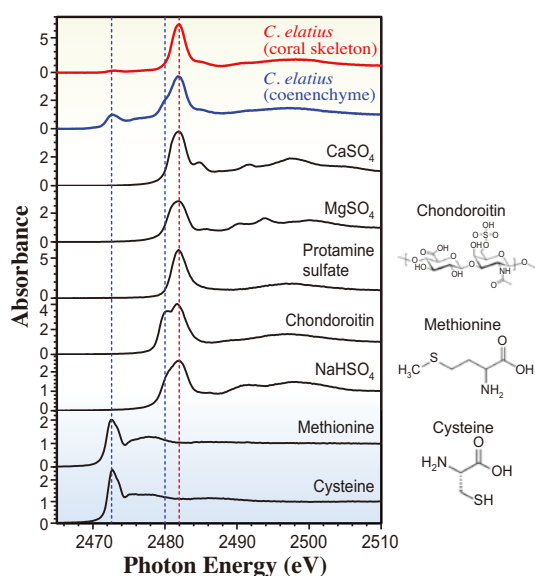


Fig. 2. XAS spectra of Japanese pink coral (*C. elatius*) and sulfur-containing material standards in the sulfur K-edge region.

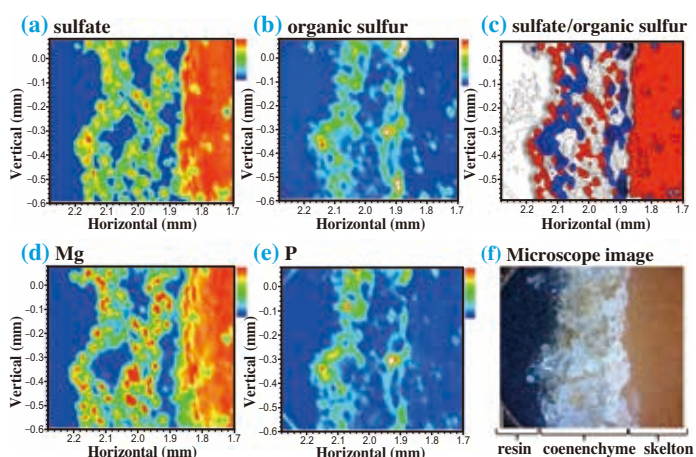


Fig. 3. Sulfur speciation and elemental mapping of Japanese pink coral (*C. elatius*) measured at the coenenchyme. Sulfur-speciation mapping at (a) 2481.9 eV (sulfate) and (b) 2472.7 eV (organic sulfur). (c) Color-coded composite image for two sulfur compounds. Red and blue areas represent areas with high concentrations of sulfate and organic sulfur, respectively. Elemental mapping images of (d) Mg and (e) P. (f) Microscope image of the Japanese pink coral skeleton for which speciation mapping is performed.

bands corresponding to growth bands. In contrast, organic sulfur compounds are mainly concentrated in the coenenchyme. The XAS analysis shows that sulfur can be incorporated into the calcite skeleton as sulfate substituting for carbonate ions, suggesting that sulfur is unsuitable as an indicator of organic matter in *C. elatius* [2]. The sulfate concentration is negatively correlated with the magnesium concentration but positively correlated with that of phosphorus, which is assumed to be mainly present in the organic matrix. The present research demonstrates that speciation analysis will contribute to a more precise understanding of trace elements in biogenic carbonates and will provide significant information on the role of trace elements in biomineralization.

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References

- [1] S.V. Smith *et al.*: Science **204** (1979) 404.
- [2] Y. Tamenori, T. Yoshimura, N.T. Luan, H. Hasegawa, A. Suzuki, H. Kawahata and N. Iwasaki: J. Struct. Biol. **186** (2014) 214.

Tungsten species in natural ferromanganese oxides related to its different behavior from molybdenum in oxic ocean

The elemental distribution at the solid/water interface is an important process in many fields of geochemistry. In marine environments, the distribution between seawater and ferromanganese oxides (FMOs), which are prevalent aggregates of Fe (oxyhydr)oxides and Mn oxides, greatly impacts the concentrations of trace elements in seawater. For example, molybdenum (Mo) is controlled by this process, since ca. 70% of its output is presumed to be incorporated into FMOs in a marine system. In addition, the isotopic composition of Mo in seawater is affected by the reaction at the seawater/FMO interface. Large isotopic fractionation of Mo occurs during adsorption on FMOs, leading to the heavier isotope of Mo remaining in seawater.

This study reveals the tungsten (W) species in natural FMOs and its difference from Mo. In contrast to the extensive geochemical attention on Mo, the geochemistry of W at the Earth's surface environment is poorly understood because of the limited number of studies on it. Revealing the difference and/or similarity in their geochemical behaviors may realize new insights in biogeochemistry fields. In modern oxic seawater, the concentration of W is much lower than that of Mo (molar ratio of Mo/W = ~1800) despite their similarity in crustal abundance and chemical characteristics (molar ratio of Mo/W in crust = ~3). This is due to the much larger enrichment of W in FMOs compared with Mo, but the origin has been a mystery for decades.

Although molecular-scale information is critical to understand the enrichment mechanism, a technical

difficulty has prevented the XAFS analysis of W in natural FMOs. For trace elements, fluorescence XAFS is conventionally conducted with an energy-dispersive detector, such as a Ge solid-state detector (Ge-SSD). However, this detector has an upper counting limit on the order of 10^5 (photons/s) due to the pulse-shaping time of the amplifier, which is on the 100-ns order. Thus, intense scattering and fluorescence from predominant elements such as Fe and Mn and/or interferences of the K lines of Ni, Cu, and Zn ($1000 \text{ mg}\cdot\text{kg}^{-1}$ - 2%) prevent high quality fluorescence XAFS spectra of W ($<100 \text{ mg}\cdot\text{kg}^{-1}$) from being obtained. To deal with this problem, we applied wavelength-dispersive XAFS to W in FMOs. Introducing a bent crystal Laue analyzer (BCLA) in front of the Ge-SSD allows the L lines of W to be selectively extracted, providing spectra with higher S/B and S/N ratios.

First, we compared the spectral quality of the W L_3 -edge XANES by the wavelength dispersive XAFS method to that by the conventional method to evaluate the effects of BCLA (Fig. 1). The XAFS measurements were conducted at BL37XU. In the conventional mode, the quality of the spectra (a) in Figs. 1(A-C) was poor mainly due to the intense interferences of Ni, Cu, and Zn. In contrast, the efficient removal of the background by the introduction of BCLA enabled us to obtain precise shape in the XAFS spectra (b) in Figs. 1(A-C). Significant improvements were due to the 13-fold increase in the S/B ratio and the 4.8-fold increase in the S/N ratio. These observations demonstrate that BCLA

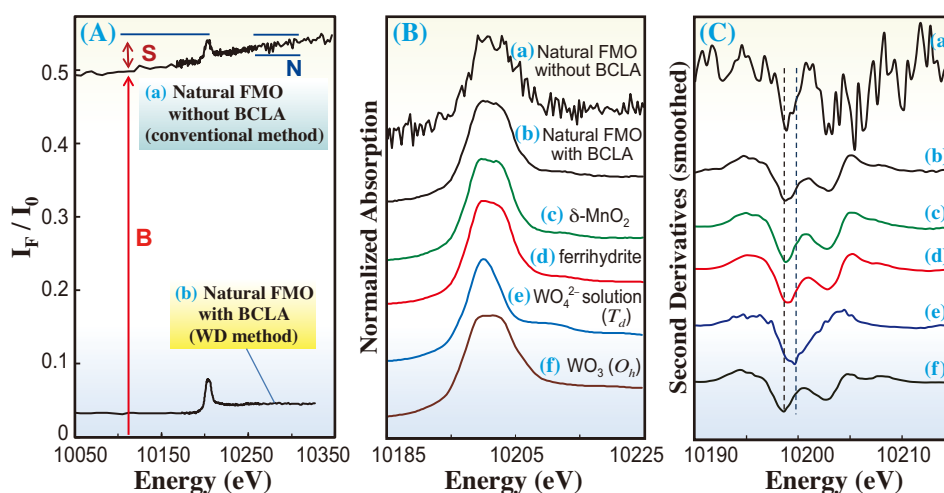


Fig. 1. Tungsten L_3 -edge XANES spectra. (A) Raw spectra, (B)-(C) after background subtraction: (B) normalized spectra and (C) their second derivatives. Natural ferromanganese oxides (a) without BCLA and (b) with BCLA. W species adsorbed on (c) δ - MnO_2 and (d) ferrihydrite. Reference compounds of (e) WO_4^{2-} solution and (f) WO_3 .

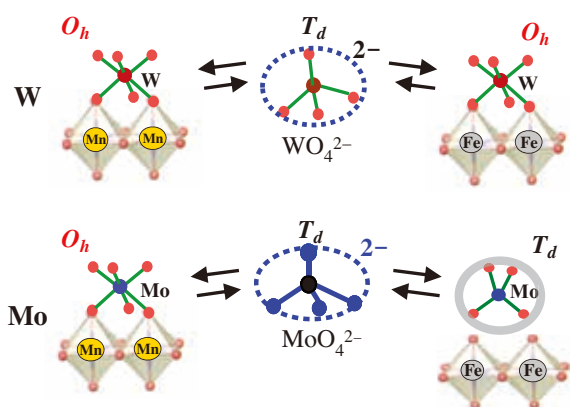


Fig. 2. Structural model of the W species adsorbed on synthetic Fe (oxyhydr)oxides and Mn oxides.

is suitable for the W L_3 -edge XAFS measurements of natural FMOs containing approximately 50 mg·kg⁻¹ of W with 1000–3500 mg·kg⁻¹ of Ni, Cu, and Zn.

As a result, we found that the W species in natural FMOs has a distorted O_h symmetry. The host phase of W in natural FMOs seems to be Mn oxides. In addition, we also revealed the structure of the W species adsorbed on synthetic Fe/Mn (oxyhydr)oxides where all the W species are inner-sphere complexes in hexavalent and distorted O_h symmetry (Fig. 2). These findings indicate that similar to the case for Mo, the negatively-charged WO_4^{2-} ion mainly adsorbs on the negatively-charged Mn oxide phase in natural FMOs via a strong chemical interaction. On the other hand, a clear difference was found in their structure on Fe (oxyhydr)oxides: inner-sphere complex of W with symmetry change from T_d dissolved species to O_h adsorbed species and the outer-sphere complex of Mo without symmetry change.

These molecular-scale insights are consistent with their macroscopic adsorption behaviors on synthetic Mn oxides and Fe (oxyhydr)oxides investigated in the laboratory (Fig. 3). For Mn oxides, the similar adsorptions between W and Mo are due to their formation of inner-sphere complexes. In contrast, the larger adsorption of W on Fe (oxyhydr)oxides is due to its formation of inner-sphere complexes compared with the outer-sphere complexes for Mo. Especially, W shows a two order of magnitude larger enrichment on Fe (oxyhydr)oxides than Mo under conditions similar to seawater (pH 8, $I = 0.70$ M). This difference is comparable to the observation of a natural seawater/FMO system. Therefore, we consider that the formation of inner-sphere complexes of W on Fe (oxyhydr)oxide phases provide a molecular explanation for the larger enrichment of W than Mo into natural FMOs, which greatly affects their concentrations in modern oxalic seawater.

In addition, isotopic fractionation of W during adsorption on Fe/Mn (oxyhydr)oxides, which has not yet been reported, is expected from the molecular symmetry. In the case of Mo, large isotopic fractionation occurs during adsorption on Mn oxides due to the structural difference between dissolved and adsorbed species. In contrast, Mo shows little or no fractionation during adsorption on Fe (oxyhydr)oxides due to the similarity of the local structure between adsorbed and dissolved species. These isotopic behaviors of Mo lead to extensive use of the Mo isotope system in paleo-environmental studies. In the case of W, the difference in the coordination number between dissolved and adsorbed species implies a striking isotopic fractionation of W during adsorption on both Fe (oxyhydr)oxides and Mn oxides. Although further studies are still necessary to elucidate the precise relationship between the structure and isotopic fractionation, the structural information indicates the potential importance of W isotope systems as a paleo-environmental proxy.

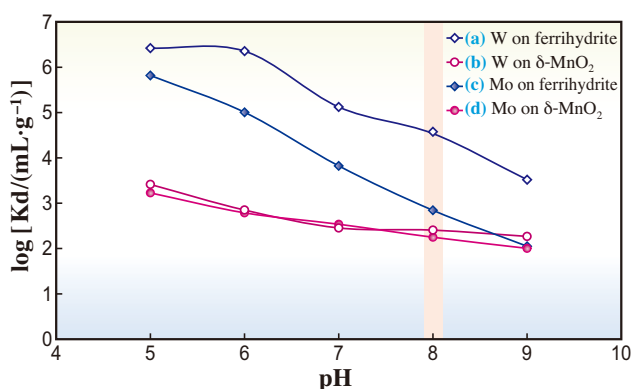


Fig. 3. Adsorption envelopes for W and Mo on ferrihydrite and δ -MnO₂ ($I = 0.70$ M, NaNO₃).

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References

- [1] J. Barling and A.D. Anbar: *Earth Planet. Sci. Lett.* **217** (2004) 315.
- [2] T. Kashiwabara *et al.*: *Chem. Lett.* **39** (2010) 870.
- [3] T. Kashiwabara, Y. Takahashi, M.A. Marcus, T. Uruga, H. Tanida, Y. Terada, A. Usui: *Geochim. Cosmochim. Acta* **106** (2013) 364.
- [4] T. Kashiwabara *et al.*: *Geochim. Cosmochim. Acta* **75** (2011) 5762.

Application of selenium in barite as a new redox indicator

The redox potential (E_h , if standardized to hydrogen electrode) is an important physico-chemical factor that controls chemical processes in the hydrosphere in the Earth, because redox reactions are closely related to the behaviors of various chemical species in water. A number of previous studies have estimated E_h based on the solid-water distribution of redox-sensitive elements reflected in the variation of the oxidation states, such as the relative enrichment-depletion profiles of particular elements (e.g., iron and manganese) in the environment. However, the estimated redox condition based on these profiles may be unreliable because (i) elemental concentrations are influenced by many factors, such as secondary adsorption-desorption reactions and diagenesis, and (ii) only the relative evaluation of the redox condition is possible by the depth profile of particular elements. Thus, the aim of this study is to propose a new redox indicator using the oxidation state of a redox sensitive element in a certain mineral to estimate directly the redox condition at the time of the mineral formation. We focused on the distribution behavior of selenium (Se) oxyanions into barite. Selenium is dissolved in water as selenate (SeO_4^{2-}) or selenite (SeO_3^{2-}) ion under oxic and suboxic conditions, respectively, and both can be fixed into the sulfate site of the crystal lattice of barite [1]. If barite incorporates both Se(IV) and Se(VI), then the Se(VI)/Se(IV) ratio recorded in barite may reflect the ratio in water, which consequently indicates the redox condition and whether barite precipitated below or above the redox boundary of Se(VI)/Se(IV).

Coprecipitation experiments of Se with barite were conducted at pH 4.0 and 8.0 to investigate the influence of the oxidation state on its immobilization into barite by the Se *K*-edge X-ray absorption near-edge structure (XANES) measured at **BL01B1** and high-performance liquid chromatography connected to inductively coupled plasma-mass spectrometry. These two pH conditions were adopted considering the typical pH values for hydrothermal water (pH = 3.0–4.0) and seawater (pH = 8.0). The oxidation state of Se in the precipitates was determined by XANES based on the fit of the sample spectra by a linear combination of the spectra of BaSeO_3 , BaSeO_4 , and native Se (Fig. 1) [2]. The results show that the Se(VI)/Se(IV) ratio in barite is primarily correlated with that of the Se(VI)/Se(IV) ratio in water at both pH's (Fig. 2), which in turn can provide physicochemical, biogeochemical, and redox information about the environment. All these results are also observed for various chemical

compositions of water and/or saturation indices of barite [2]. These laboratory experiments suggest that the barite-selenium oxyanion system can be used as a reliable redox indicator to estimate the Se(VI)/Se(IV) ratio in water. If the system is under equilibrium in terms of the redox condition, E_h can be estimated based on the Se(VI)/Se(IV) ratio.

In a natural system, the distribution behaviors of ions between mineral and water are influenced by several factors, such as ionic strength, precipitation rate, etc. [3]. Thus, natural barite samples in marine sediment collected near the hydrothermal vent system in the Izena Cauldron, Okinawa Trough were also analyzed by micro-XRF and XANES to investigate the applicability of the barite-selenium system as a redox indicator in natural systems (**BL37XU**) [4]. Micro-XRF elemental mapping shows that the distribution of Se is correlated with that of Ba, especially in the left part in Fig. 3, suggesting that Se is incorporated into barite within the sediment. Micro-XANES analysis was performed at a spot with a relatively high concentration of Se in the barite; Se is mainly incorporated as Se(IV) in this marine sediment (Fig. 3 (E)). Based on the aforementioned laboratory experiments barite precipitates under suboxic condition where Se is dissolved as Se(IV). This is consistent with the fact that the pore water of the sample is under a mildly reducing condition ($E_h = 150$ mV at pH 7.1) where Se(IV) can be the main Se species in the water. Thus, the good correlation between laboratory experiments and natural

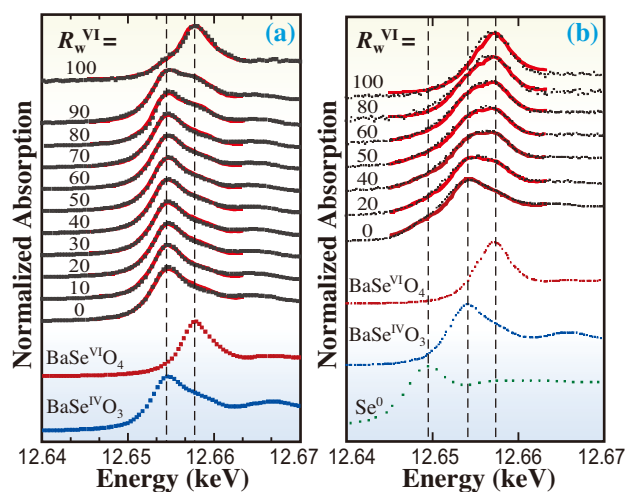


Fig. 1. Normalized Se *K*-edge XANES spectra of the reference materials (BaSeO_3 , BaSeO_4 , and native Se) and Se in barite at (a) pH 8.0 and (b) pH 4.0 as a functions of R_w^{VI} (= Se(VI)/total Se).

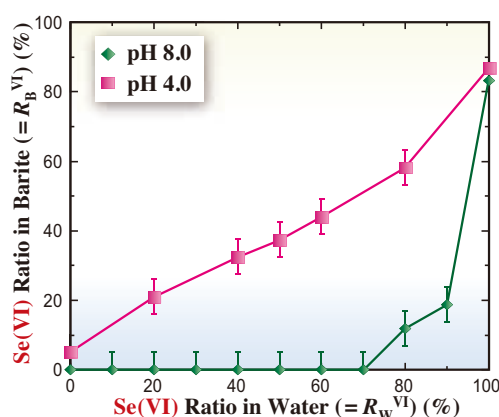


Fig. 2. Relationship between R_w^{VI} and R_b^{VI} . Se(VI)/Se(IV) ratios in barite and in solution are measured by XANES and HPLC-ICP-MS, respectively.

analysis suggests that the Se(VI)/Se(IV) ratio in barite can be used to estimate roughly the redox condition of the coexistent water during the formation of barite.

Application of this indicator has several unique characteristics. First, the method is sensitive to the redox condition between the high and low E_h regions as indicated by the MnO_2/Mn^{2+} and $Fe(III)/Fe^{2+}$ boundaries, respectively. Second, this method can be used to estimate the redox condition based on the

Se(VI)/Se(IV) ratio using one barite sample, whereas using other data to evaluate the redox condition requires various geochemical data, such as the depth profile of redox-sensitive elements. This method has many potential applications, including (i) to estimate the redox condition when barite was precipitated and (ii) to estimate the redox condition in an environment where a Pt electrode cannot be used to measure E_h such as in hydrothermal water.

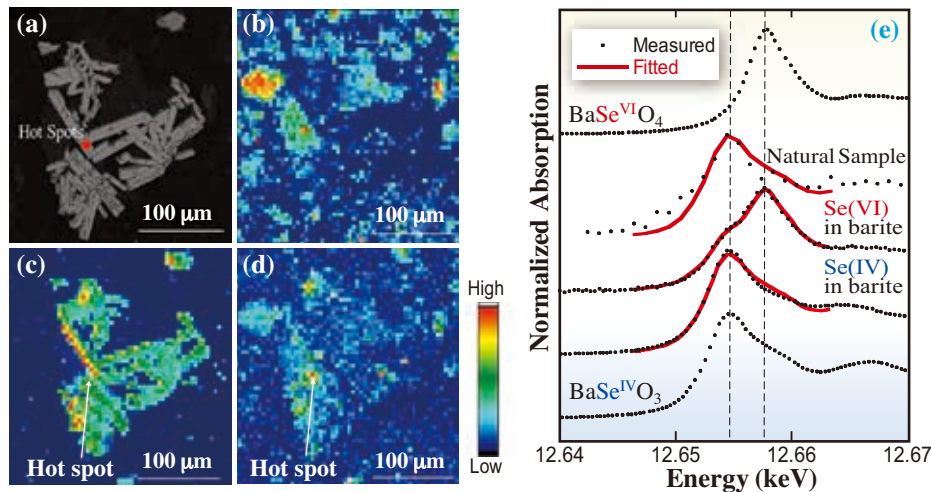


Fig. 3. BSE and μ -XRF images and μ -XANES of the sample. (a) BSE image of barite. (b, c, d) μ -XRF images of Fe, Ba, and Se, respectively. (e) Normalized Se K-edge XANES of Se reference materials ($BaSeO_3$ and $BaSeO_4$), Se in barite at pH 8.0 added as Se(IV) or Se(VI), and Se in natural barite at hot spots.

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References

- [1] A.J. Andara *et al.*: Cryst. Growth Des. **5** (2005) 1371.
- [2] K. Tokunaga, Y. Yokoyama and Y. Takahashi.: Geochem. Geophys. Geosyst. **14** (2013) 4826.
- [3] R.B. Lorenz: Geochim. Cosmochim. Acta **45** (1981) 53.
- [4] K. Tokunaga, Y. Yokoyama and Y. Takahashi: Chem. Lett. **42** (2013) 1068.

Determining the operating principle of a three-terminal domain wall device

The rapid progress in magneto-resistive random access memory (MRAM) technologies provides non-volatile devices with high-speed operations, low power consumption, and high reliability. MRAM cells are based on magnetic tunnel junctions (MTJs) consisting of two ferromagnetic layers separated by a thin insulating layer. Parallel and antiparallel magnetic configurations of the MTJ induce low and high resistance states, which are defined as R_0 and R_1 , respectively, due to the tunnel magnetoresistance (TMR) effect. Magnetization switching by the electric current through the MTJ or the ferromagnetic layer, such as spin-injection magnetization reversal [1] or current-induced domain wall (DW) motion [2], can be applied to write data in MRAM cells. Electrical manipulation of DWs is one candidate technique to write data to three-terminal MRAM cells with separate write and read current pathways.

Previously, we reported the development of a three-terminal DW device [3]. A low-current writing operation at 0.16 mA and a TMR of 80% were obtained for a 130-nm-wide free layer. The free layer, which is comprised of a CoFeB layer, with MgO capping and Ta seed layers, has a perpendicular magnetic anisotropy (PMA) and an excellent read property with a high TMR ratio [4]. However, the principle to write data in the DW device is unclear. An understanding of the operating principle is indispensable to further reduce the write current. In this article, the mechanism responsible for writing data in the device is derived from direct observations of DW motion in a Ta/CoFeB/MgO wire by means of photoemission electron microscopy with X-ray magnetic circular dichroism (XMCD-PEEM) and the electrical characteristics of the three-terminal DW device [5].

Figure 1(a) schematically depicts our three-terminal DW device, which are comprised of a free layer (FL), hard magnets (HMs), and a reference layer (RL). HMs (HM1 and HM2) with PMA are attached under each end of the FL. HM1 and HM2 were fabricated separately before deposition of the MTJ. The 5-nm Ta capping layer serves as the spacer between the FL and each of the HMs. The direction of magnetization in the sections of the FL over HM1 or HM2, which are referred to as fixed regions 1 and 2, follows the direction of the magnetization in each HM due to the stray field from each HM. Applying the appropriate external magnetic fields produces a single DW at the boundary between fixed region and free region. Figure 1(b) demonstrates typical results for R_{MTJ} vs. write current at room temperature. We then injected 200-ns current pulses

into the FL and observed respective transitions of R_{MTJ} from R_0 to R_1 and from R_1 to R_0 when the direction of the current was positive and negative, respectively. Here, the pulsed current passing from HM1 to HM2 was defined as $+I$. The insets of Fig. 1(b) show that the DW appears to be moving in the direction of the current flow, if we simply assume that the current pulses are driving a DW between the boundaries of the free region and fixed regions. To clarify the operating principle of the three-terminal DW device, we investigated the characteristics of current-induced DW motion in a stacked structure identical to that of the FL.

The magnetic domains in the Ta/CoFeB/MgO wire as illustrated in Fig. 2(a) were directly observed by XMCD-PEEM at beamline BL25SU. The photon energy was set to the Fe- L_3 absorption edge (708.4 eV) to obtain a contrast between magnetic domains. Observed samples 1 and 2 were prepared as follows. After applying $H = +2$ kOe to align the magnetization in a CoFeB wire, the DW was introduced at the left edge of the Hall crossing by injecting current into electrode A with $H = -100$ Oe (for both samples). For sample 2,

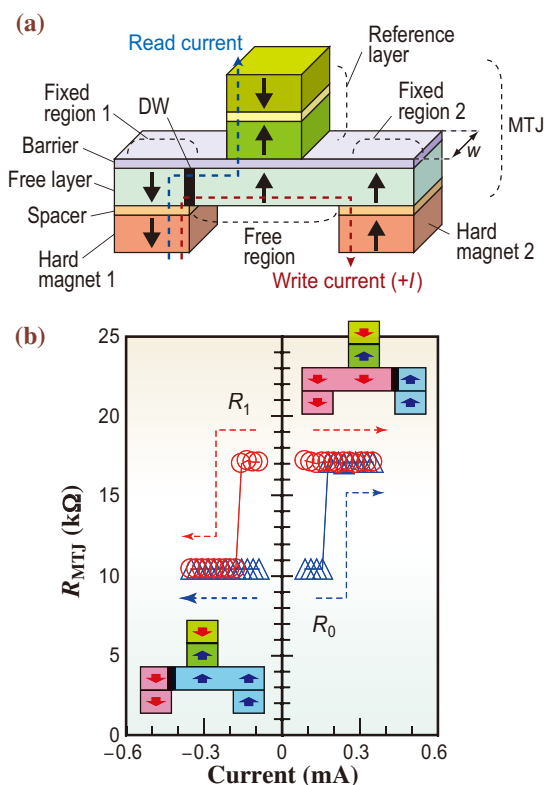


Fig. 1. (a) Schematic illustration of the three-terminal DW device. (b) Typical plot of R_{MTJ} vs. 200-ns current pulses at room temperature for a 130-nm-wide free layer. Inset shows magnetic configurations in the R_0 and R_1 states.

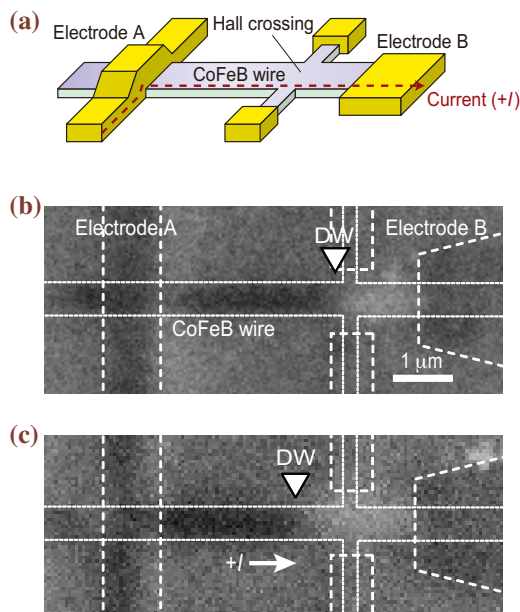


Fig. 2. (a) Schematic illustration of the device for XMCD-PEEM observations. CoFeB wire has a Hall crossing for electrical detection of DW motion, and two electrodes (labeled electrode A and B) are attached to it. (b) PEEM image of sample 1 taken after introducing a DW and (c) PEEM image of sample 2 taken after injecting a single 80-ns pulse of current $I = +0.16$ mA into the CoFeB wire. Darker and brighter regions in the CoFeB wire correspond to downward and upward magnetization, respectively. Dashed white lines indicate edges of the CoFeB and electrodes, and positions of DW by the white inverted triangles denote DW positions.

a single 80-ns current pulse of +0.16 mA was injected into the CoFeB wire. Figures 2(b) and 2(c) show PEEM images of sample 1 and 2, where the DW positions are approximately 100 and 700 nm from the left edge of the Hall crossing, respectively. Comparing the positions of the DWs in Figs. 2(b) and 2(c) clearly confirms that the motion of the DW is against current flow. Therefore, the assumption that the existing DW is driven in the direction of current flow is inconsistent with the explanation for magnetization switching in Fig. 1(b).

In response to the XMCD-PEEM results, we performed further analyses of the electrical characteristics in the DW device combined with theoretical calculations [5], and found the nucleation of DW while injecting a write current due to the influence of the stray field from each HM. The principles behind the operation of our device were depicted in Fig. 3. After preparing a DW at the boundary between the free region and fixed region 1, a positive write current is injected from HM1 to HM2. This leads to domain nucleation, which produces DW_{nc1} and DW_{nc2} around the left edge of HM2. DW_{nc1} is relatively free of the effects of stray field from HM2. Accordingly, only DW_{nc1} is driven toward the

direction of HM1, which is against the current flow. This eliminates the domain between DW_{nc1} and the original DW at the boundary between the free region and fixed region 1. Consequently, DW_{nc2} is the only remaining DW. Subsequently injecting a negative write current from HM2 to HM1 causes two new DWs labeled DW_{nc3} and DW_{nc4} to be nucleated around the right edge of HM1. While injecting the current, only DW_{nc3} is driven toward the direction of HM2 and annihilates with DW_{nc2} in a similar fashion.

In summary, the switching of magnetization in our three-terminal DW device is due to nucleation of DW and its displacement against current flow. XMCD-PEEM provides conclusive evidence of DW motion, which has helped clarify the operating principle.

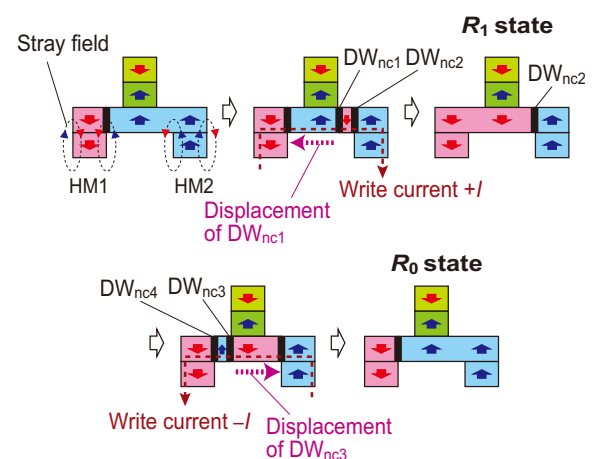


Fig. 3. Process of magnetization switching in our three-terminal DW device. DW_{ncp} ($p = 1$ to 4) denotes the DWs nucleated by the injection of positive and negative write currents. Position of DW nucleation is attributed to the direction of the stray field from each HM and magnetization in the free region.

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References

- [1] Y. Huai *et al.*: Appl. Phys. Lett. **84** (2004) 3118.
- [2] A. Yamaguchi *et al.*: Phys. Rev. Lett. **92** (2004) 077205 [Errata: **96** (2006) 179904].
- [3] T. Suzuki *et al.*: Symp. VLSI Technology Dig. Tech. Pap., 2013, p. 138.
- [4] S. Ikeda *et al.*: Nat. Mater. **9** (2010) 721.
- [5] H. Tanigawa, T. Suzuki, K. Suemitsu, N. Ohshima, T. Kitamura, T. Ohkoshi, M. Kotsugi, T. Kinoshita, T. Koyama, D. Chiba, Y. Yoshimura, K. Ueda, T. Ono and E. Kariyada: Jpn. J. Appl. Phys. **53** (2014) 063002.

Structural characterizations of organic semiconductor thin films investigated by two-dimensional grazing incidence X-ray diffraction

Organic thin-film transistors (OTFTs) have attracted much attention, and their performances have been continuously improved. It is well known that the structural properties, such as crystallinity, grain size, crystal orientation and polymorphism determine the carrier transport properties in OTFTs [1]. Hence, crystallographic characterization of thin organic films is necessary to further improve the performances of OTFTs.

Grazing incidence X-ray diffraction (GIXD) has been used in the structural analysis of organic thin films. To date, the structure of organic thin films has yet to be thoroughly characterized; only the lattice parameters of the thin films have been compared to the bulk by assuming that they have similar structures. However, the crystal structures of some organic thin films differ from the bulk structures, as observed in the polymorphs of pentacene [2]. Because the crystal structure of organic semiconductors affects the carrier transport properties, a method to analyze crystal structures of thin-film organic semiconductors is necessary to understand the physical properties of OTFTs. In this paper we report the crystal structure of vacuum deposited organic thin films by two-dimensional GIXD (2D-GIXD) using a synchrotron X-ray source and a high-sensitivity area detector (PILATUS 300K) [3].

The 2D-GIXD measurements were carried out using a multi-axes diffractometer combined with PILATUS 300K at BL46XU beamline. The geometry of 2D-GIXD measurement is depicted in Fig. 1. α, ω -Hexyl-distyryl-bithiophene (DH-DS2T, see the molecular structure in Fig. 2(a)), a p-type organic semiconductor, was chosen as the sample [4]. The crystal structure of DH-DS2T has not yet been clarified since it is difficult to grow a

single crystal. DH-DS2T was vacuum deposited onto a silicon wafer coated with native oxide (SiO_2) at room temperature.

The 2D-GIXD pattern from the polycrystalline DH-DS2T thin film is shown in Fig. 2(b), where $2\theta_z$ on the vertical axis and $2\theta_{xy}$ on the horizontal one denote the takeoff and horizontal component of the scattering angle, respectively. A number of diffraction spots were successfully observed owing to the combination of a sensitive 2D-detector and a high-brightness synchrotron X-ray source. The observed pattern indicates that the sample is a highly oriented polycrystalline film along the surface normal.

As the primary step for structure analysis of the 2D-GIXD pattern, the unit cell parameters and crystalline orientation were determined from the diffraction positions. Figure 2(b) shows the comparison of experimental and calculated (marked by open circle) 2D-GIXD patterns. All the observed diffractions can be indexed by a monoclinic unit cell with the following lattice parameters: $a = 0.58$ nm, $b = 0.78$ nm, $c = 3.43$ nm, $\alpha = \gamma = 90.0^\circ$, and $\beta = 94.3^\circ$. It was found that the c^* -axis of the unit cell is oriented perpendicular to the substrate surface, although the azimuthal orientation is random in the polycrystalline film. Some of the main diffractions are indexed and labeled in the figure. The c value of DH-DS2T is larger than that of the DS2T thin film, which lack alkyl chains ($a = 0.77$ nm, $b = 0.56$ nm, $c = 2.08$ nm, and $\beta = 95.3^\circ$) [5]. Moreover, parameters a and b of DH-DS2T are similar to the exchanged parameters b and a of DS2T, indicating that the tilting direction of c -axis against the a - b plane is changed from long axis to short axis upon attaching alkyl chains.

As the second step for structural analysis, the inner structure of the DH-DS2T unit cell was considered by comparing the observed intensities with the calculated ones. As a model for calculation, the DH-DS2T molecule was divided into two parts: DS2T and alkyl parts, as shown in Fig. 2(a). Three rotation angles (θ_t , φ_t and ϕ_t), which were used to define better configurations of the molecules in the unit cell, can describe suitable atomic positions (r_j). The molecular structure factor of the DH-DS2T crystal in a thin film is calculated using these rotation angles and the space group. The calculated and observed intensities agree well, and the extinction rules at $\theta_t = 25^\circ$ correspond well. Figure 3 shows the three-dimensional crystal structure of the DH-DS2T thin film obtained from the best-fit of r_j . Our calculation elucidates that the overall DH-DS2T molecule in the unit cell tilts about 25° against the substrate normal.

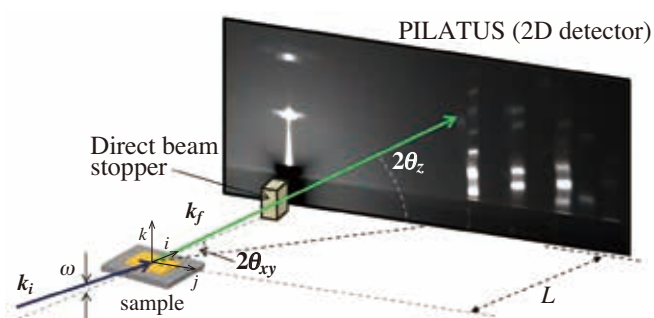


Fig. 1. Geometry of the 2D-GIXD measurement setup. i , j , and k are defined as the unit vector. Element of i vector is parallel to the incident beam. [3]

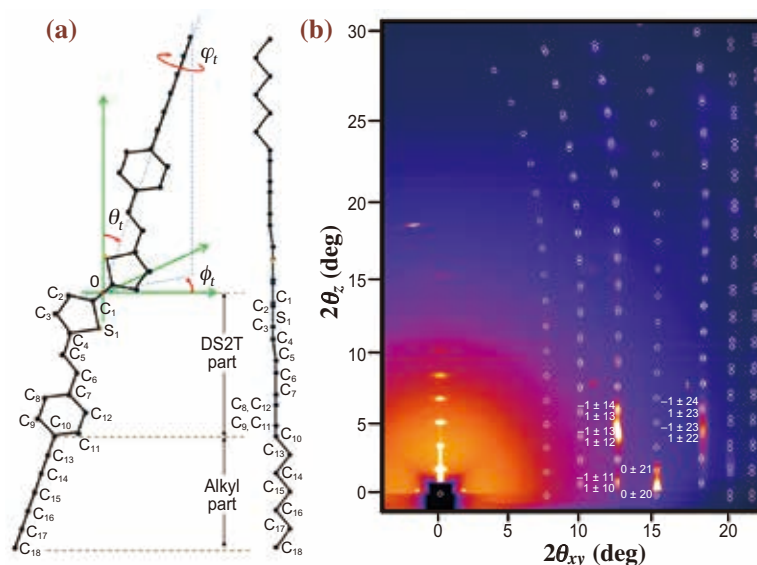


Fig. 2. (a) Molecular structure of DH-DS2T. DH-DS2T molecule was divided into two parts for the calculation: DS2T and the alkyl parts. (b) 2D-GIXD pattern image of a DH-DS2T thin film on SiO₂. Black square on the bottom-left corner is the shadow image of the direct-beam stopper. Open circles show the results of simulated diffractions. [3]

This means that the inner structure of the DH-DS2T unit cell is partly similar to that of DS2T.

A number of the X-ray diffraction spots from the highly oriented DH-DS2T thin film were simultaneously observed using a combination of the sensitive 2D-detector and the high-brightness synchrotron X-ray

source. The method developed in the present study can be widely applied to other highly oriented crystalline organic materials and will contribute to clarify their crystal structures. The method is especially vital for materials that form extrinsic structures in thin films or cannot be grown into bulk single crystals.

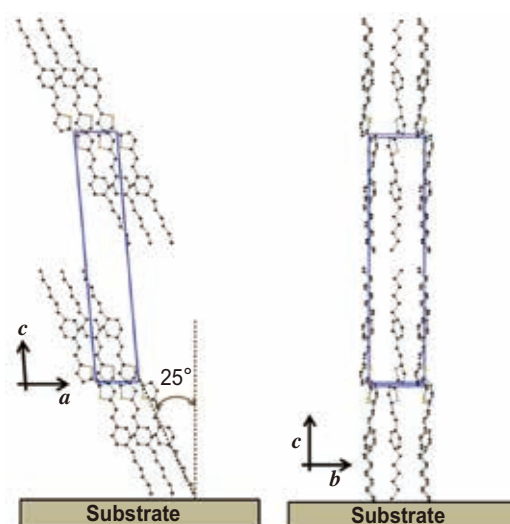


Fig. 3. Three-dimensional crystal structure of the DH-DS2T thin film determined by 2D-GIXD pattern. [3]

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References

- [1] H. Yanagisawa *et al.*: Thin Solid Films **464** (2004) 398.
- [2] H. Yoshida *et al.*: Appl. Phys. Lett. **90** (2007) 181930.
- [3] T. Watanabe, T. Koganezawa, M. Kikuchi, C. Videtot-Ackermann, J. Ackermann, H. Brisset, I. Hirose and N. Yoshimoto: Jpn. J. Appl. Phys. **53** (2014) 01AD01.
- [4] C. Videtot-Ackermann *et al.*: Org. Electron. **9** (2008) 591.
- [5] M. Ito *et al.*: Mol. Cryst. Liq. Cryst. **491** (2008) 264.

Latest findings and measures in hair changes due to aging

Several basic scientific approaches targeting aging are being undertaken by cosmetic companies to meet the demands arising from demographic changes in our country. How Japanese cosmetic companies are addressing trends in our aging society is being observed by cosmetic companies around the globe.

In a survey of beauticians and internal hair evaluators who deal with hair on a daily basis, “fluffy” stood out as words used to describe age-associated changes in hair. Because we hypothesized that the reduced internal density of hair is responsible for these changing perceptions, we measured the internal density (g/cm^3) of hair samples from subjects of various ages at their newly formed (healthy hair) and damaged parts (damaged hair, approximately 30 cm away from the newly formed part). The results showed that newly formed hair grows at a lower density as age advances and that the density in the damaged parts is less than that in the newly formed parts regardless of age (Fig. 1(a)). Furthermore, the density ratio in the newly formed parts to that in the damaged parts decreases with age (Fig. 1(b)). This indicates that the age-associated reduction in hair density is greater in the damaged parts compared with that in the newly formed parts; we call this phenomenon of an age-associated decrease in hair density “Hair-porosis” in an analogy to osteoporosis. Currently, we presume that this phenomenon is a combination of an intrinsic change in hair (i.e., proteins, which account for approximately 80% of hair constituents, are more readily eluted from hair at an advanced age [1]) and changes in the frequency of cosmetic routines such as hair dyeing. Future detailed studies should confirm these presumptions.

To investigate the structural changes that occur within hair due to density changes, we observed cross-

sections of hair with TEM and found that the frequency of internal voids in hair increases with aging. Because damage during sample preparation, such as when cutting the cross-sections, could not be eliminated, we employed the non-destructive X-ray CT method using beamline **BL24XU** to observe the internal structure of hair. This method utilizes X-ray microscope optics to achieve a sub-micrometer spatial resolution. Using 7 keV X-rays, we observed an age-associated increase in void frequency, a typical example is shown in Fig. 2. We have yet to identify the damaged portions, but hope to gain further insights by improving the spatial resolution and sensitivity for density [2].

It is generally known that hair proteins are lost due to beauty treatments, such as hair coloring and permanent wave treatments. The keratin protein has been well studied as a repair material for hair damage caused by these treatments. However, conventionally prepared keratin is easily eluted from hair by repeated washing and drying during daily care. To solve this problem, S-carboxymethyl alanyl disulfide keratin (CMADK) has been developed to meet the expectations of a long-lasting form of the keratin protein [3].

We verified whether continued use of CMADK can improve the age-associated decrease in density using hair samples from subjects in their 40s. The samples were treated with a 1% CMADK solution, washed, and dried using a dryer; this process was repeated for a designated number of days. The internal density of the samples was measured daily. The result showed that the continued use of CMADK gradually improved the internal density of hair (Fig. 3(a)). We then incorporated CMADK into a commercial product and demonstrated that the product effectively improved the internal density of hair when used daily as described

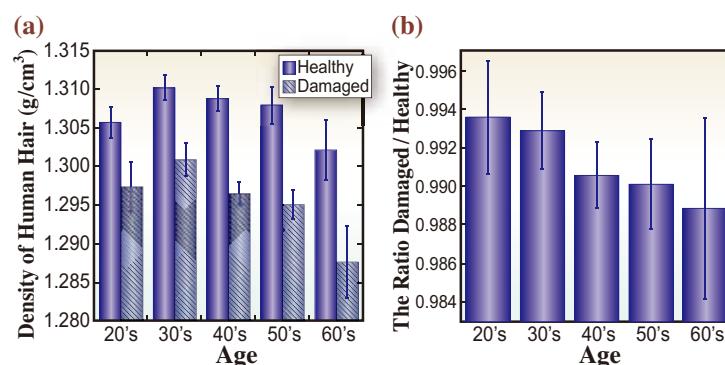


Fig. 1. Density of healthy and damaged hair with age (N = 219). Healthy: hair at the base ($P < 0.01$); Damaged: hair 30 cm from the base ($P < 0.004$).

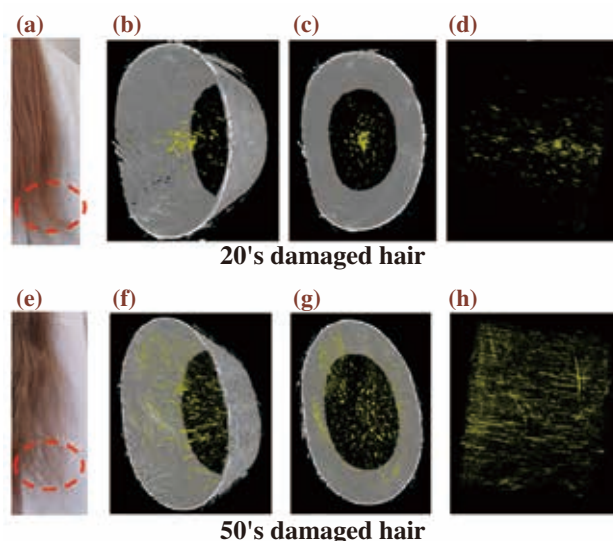


Fig. 2. 3D images of human hair using X-ray CT method at beamline BL24XU.

above (Fig. 3(b)). We have successfully developed a series of products incorporating a haircare system to recover the internal density of hair, which would be otherwise lost with age (Fig. 3(c)). We hope to

continue elucidating new phenomena by cutting-edge analysis and measurement technologies, and applying them to product development in order to meet diversified client needs.

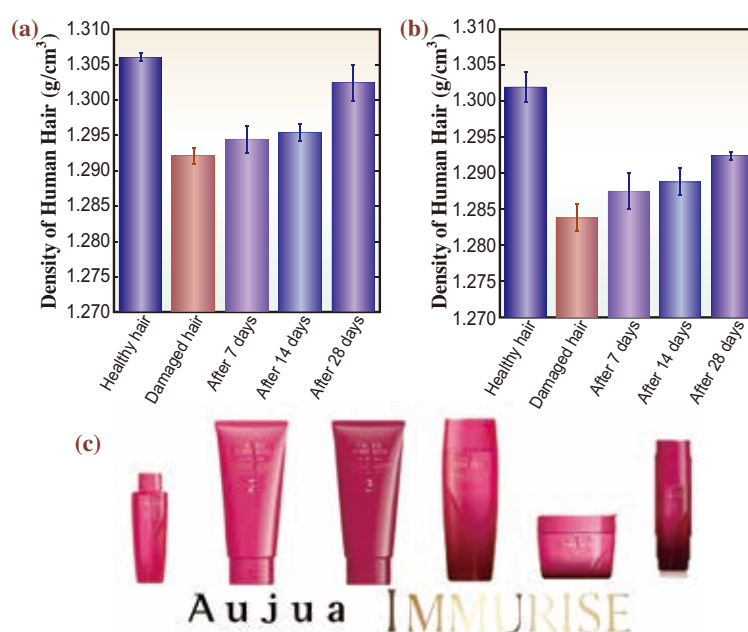


Fig. 3. Verification of the effect and products. (a) Changes in the internal density of hair when CMADK as a raw material is continuously used. (b) Changes in the internal density of hair when a CMADK-containing shampoo and conditioner are used daily, and (c) photograph of the product line.

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References

- [1] L. Ito, H. Kazumori, A. Baba, K. Suzuta: *Fragrance J.* **40** (2013) 18. (in Japanese)
- [2] H. Takano *et al.*: *Jpn. J. Appl. Phys.* **52** (2013) 040204.
- [3] M. Yoshida *et al.*: *Journal of SCCJ* **48** (2014) 190. (in Japanese)

Non-platinum group metal catalysts of direct hydrazine anionic membrane fuel cells for automotive applications

A running test of a brand new fuel cell vehicle (FCV), which utilized synchrotron analysis for its development, was carried out at SPring-8 in September 2013 (Fig. 1). The power train of the vehicle, a non-platinum group metal (non-PGM) direct hydrazine fuel cell system, was designed compactly and stored under the flat floor of the vehicle.

A fuel cell can transform chemical energy into electrical energy efficiently. In a historically famous example British chemist Sir William Robert Grove successfully generated power for the first time in 1839 by supplying hydrogen and oxygen to two platinum electrodes in sulfuric acid [1].

For automotive applications, a polymer electrolyte membrane (PEM) fuel cell is recognized as a promising power source. Figure 2 shows the key concept of our research to realize a practical non-PGM fuel cell vehicle, which is called the Creation of Anionic Fuel-cell for the Earth, or CAFE for short. An anion exchange membrane fuel cell vehicle has many advantages suitable for widespread use. Since precious metals, such as platinum, are not needed at all, this system is very advantageous with respect to resources and economic considerations. Moreover, a liquid fuel, hydrazine hydrate ($\text{N}_2\text{H}_4 \cdot \text{H}_2\text{O}$), can be supplied safely using a handy polyethylene tank as well as existing infrastructure. Additionally, liquid fuel is used as a “medium” of hydrogen and electrons to realize a hydrogen society.

The principle of the anion exchange membrane fuel is compared with the proton type fuel cell in Fig. 3. Because many active transition metals dissolve in strong acidic environments, alkaline environments are better suited for non-PGM catalysts. Moreover, oxidation of a fuel is more reactive in an alkaline environment.



Fig. 1. Pt-free, zero-emission vehicle powered by a liquid-feed fuel cell.



Fig. 2. Key concept of the CAFE project to realize a practical non-PGM fuel-cell vehicle. Illustrated by Anna Tanaka.

A liquid fuel such as hydrazine hydrate is preferable as a fuel for automotive applications due to its higher energy density and handling ease. The theoretical potential (E^0) of hydrazine (N_2H_4) is as high as 1.61 V. Thus, it is expected to contribute to down-sizing because the number of cells can be decreased. Hydrazine hydrate theoretically produces nitrogen and water by their electro-oxidation reactions without CO_2 exhausts.

Metal-nitrogen-carbon type Co polypyrrol on carbon (CoPPyC) electrocatalysts have been investigated for anion-exchange membrane fuel cells [2]. Specifically, CoPPyC oxygen reduction reaction (ORR) electrocatalysts were analyzed with synchrotron X-ray radiation because it is difficult to analyze mono cluster metal center catalysts such as macrocycle materials using lab instruments. We collaborated with Dr. Nishihata's group of JAEA, Prof. Atanssov's group of the University of New Mexico, and Prof. Kasai's group of Osaka University to establish the structure-to-property correlations.

CoPPyC was synthesized from cobalt nitrate

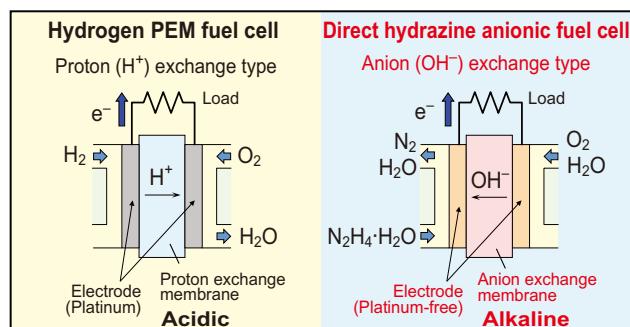


Fig. 3. Comparison of conventional fuel cell and Daihatsu fuel cell.

and PPyC. CoPPyC-A was treated in 1 M sulfuric acid to remove cobalt oxide species from CoPPyC. CoPPyC-PA was pyrolyzed and treated in 1 M sulfuric acid from CoPPyC.

In situ X-ray adsorption fine structure (XAFS) measurements at the Co *K*-edge were carried out at **BL14B2**. Oxygen-saturated alkaline electrolyte was circulated through the cell. During *in situ* XAFS analysis, potential was set at 0.1 V, −0.2 V, and −0.4 V vs. Hg/HgO reference electrode (Fig. 4(a,b)).

CoPPyC-A shows the highest coordination number initially, but after supplying an alkaline electrolyte, the coordination number and the distance to the nearest neighbor decrease. For CoPPyC-A catalyst, the coordination number decreases as the potential decreases, suggesting that the adsorbed species are removed from Co. For CoPPyC-PA catalyst, at a high potential (0.1 V), the coordination number increases from initial state, indicating that OH adsorbs stronger than O₂. As the potential decreases, so does the coordination number.

Hard X-ray photoelectron spectroscopy (HAXPES) measurements were carried out at **BL46XU** and **BL47XU** (Fig. 4(c,d)). The source X-ray energy was 7940 eV. Although the C 1s, N 1s, and O 1s, and Co 2p spectra were acquired, here only the N 1s region is shown. CoPPyC-A and CoPPyC-PA show quite

different spectral shapes: nitrogen is mainly pyrrolic in the former but pyridinic in the latter. Conversion of pyrrolic nitrogen to pyridinic occurs during pyrolysis.

The spectroscopic characterization demonstrates that the Co atom is mainly bound to pyrrolic and pyridinic nitrogens in the CoPPyC-A and CoPPyC-PA catalysts, respectively. The structure was optimized by DFT calculations. The Co-pyrrolic has a non-planar configuration, which originates from the rotational degrees of freedom along its Co–N bond and the dominant repulsive character induced between hydrogen atoms of the pyrrol trimers facing each other.

In the 20th century, fossil fuel greatly served as energy resources, and precious metals were utilized as materials of environmental protection. However, to secure these resources, territorial invasions were repeated and peace was spoiled at some locations on Earth. On the other hand, electrical energy can be generated all around the world. By confining this electrical energy in a liquid through a chemical reaction, it becomes an easy-to-use fuel, which can be used to drive a bright future with platinum-free and zero-emission vehicles. However, there are many issues that should be solved in our project, but further technical developments should be accelerated by research using synchrotron radiation.

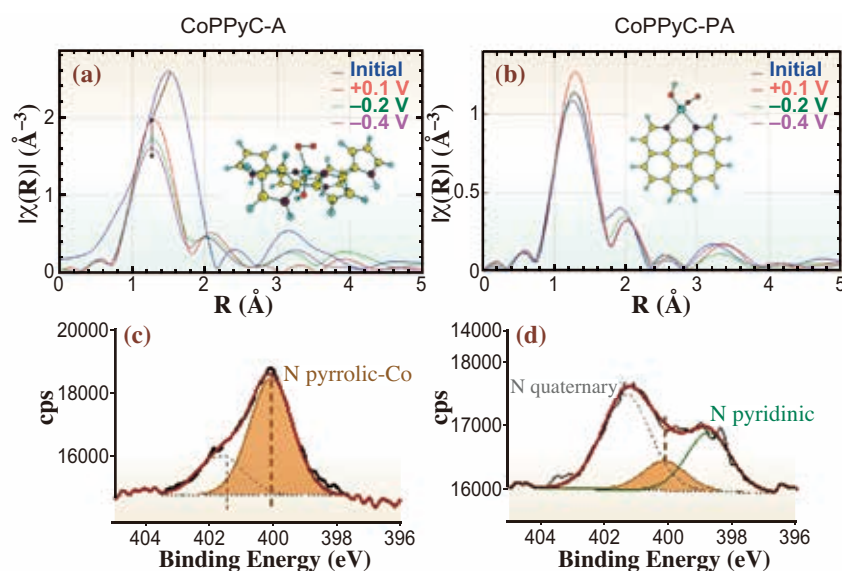


Fig. 4. Structure of CoPPyC-A and CoPPyC-PA catalysts from *in situ* XAFS and HAXPES analysis.

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References

- [1] W.R. Grove: *Philos. Mag. Ser. 3* **14** (1839) 127.
- [2] K. Asazawa *et al.*: *Angew. Chem. Int. Edit.* **46** (2007) 8024.
- [3] K. Asazawa, H. Kishi, H. Tanaka, D. Matsumura, K. Tamura, Y. Nishihata, A.G. Saputro, H. Nakanishi, H. Kasai, K. Artyushkova and P. Atanassov: *J. Phys. Chem. C* **118** (2014) 25480.

Backward-angle photoproduction of ω and η' mesons from protons at $E_\gamma = 1.5 - 3.0$ GeV

A linearly polarized photon beam with an energy of a few GeV can be produced by backward-Compton scattering (BCS) from head-on collisions between laser photons and 8-GeV electrons in the storage ring. Photon beam in the energy range from 1.5 to 3.0 GeV have been utilized for nuclear physics experiments at beamline **BL33LEP**. LEPS experiment has been performed to study nuclear science using 355-nm and deep-UV 257-nm lasers to produce Compton-scattered photons in the range of 1.5 to 2.4 GeV and 1.5 to 3.0 GeV, respectively.

Although many nucleon resonances have been identified using pion beam experiments, many resonances predicted by the constituent quark model remain to be discovered. This dilemma is known as the “Missing resonance problem”. Some of these missing resonances may not be observed in pion beam experiment due to weak coupling with the pion. Therefore, measurements of meson production with a photon beam provide a complementary tool to study nucleon resonances. Recently, differential cross section and polarization variable of η , η' and ω mesons have been measured in experiments with large acceptance spectrometers such as CB-ELSA, GRAAL and CLAS. Partial wave analysis (PWA) of their results indicates a significant contribution from nucleon resonances in the differential cross section of meson photoproduction at large scattering angle ($\Theta_{CM} \sim \pi/2$) at $\sqrt{s} \sim 2$ GeV, although the list of resonances depends on models.

At backward angles in the center of mass system, the contribution from the baryon exchange (u -channel) should become significant. In general, the differential cross section from the baryon exchange is much smaller than that from the meson exchange, which has a significant contribution at forward angles. On the other hand, the angular distribution of mesons from nucleon resonances could have a rapid change at forward and backward angles when the nucleon resonances have high angular momenta. The contribution of nucleon resonances with high angular momenta tends to be stronger at forward and backward angles than at intermediate angles. Therefore, the differential cross section at backward angles is sensitive to nucleon resonances with high angular momenta and becomes a good tool to identify and search these resonances.

A new measurement was carried out at BL33LEP with a time projection chamber (TPC) to detect the decay products. We used a liquid hydrogen target. Production of ω and η' mesons was clearly identified by detecting protons at the LEPS forward spectrometer and pions at the TPC. In addition, the incident photon energy, E_γ , was extended to 3.0 GeV by using a new deep-UV laser.

The photoproduction of the ω and η' mesons were measured via the following decay modes:

$$\gamma p \rightarrow p \omega \rightarrow p \pi^+ \pi^- \pi^0$$

$$\gamma p \rightarrow p \eta' \rightarrow p \pi^+ \pi^- \eta$$

Protons were measured by the LEPS forward spectrometer. Charged pions were detected in the TPC. π^0 or η mesons were identified by the missing mass information to select the above reactions. The events generated in the target were selected by the determined position of the reaction point. The acceptance was evaluated using a Monte-Carlo simulation. The yields of detected ω and η' mesons were corrected by the number of photon, the number of target protons, and the efficiency to determine the differential cross sections.

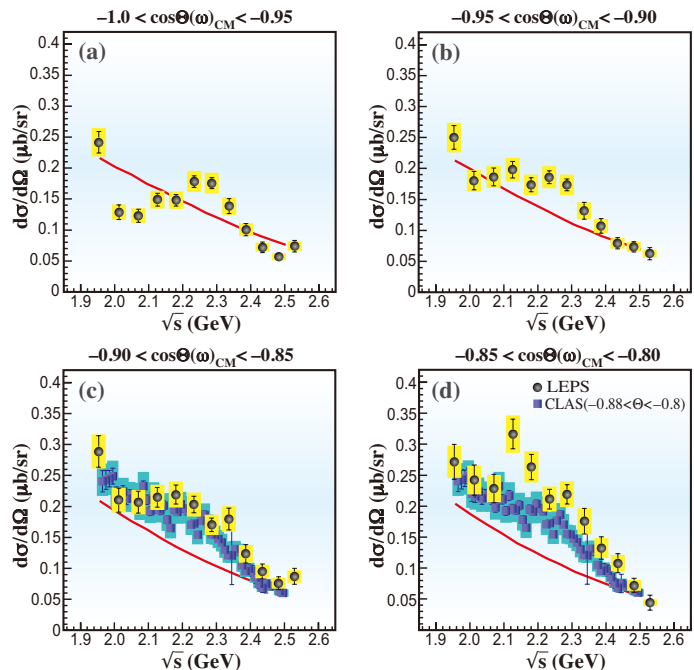


Fig. 1. Differential cross sections for ω photoproduction as a function of $\sqrt{s}$. Black circles are the present results [1]. Shaded bars represent the systematic uncertainty. Blue squares are the CLAS results [2]. Smooth lines represent the theoretical calculation [3].

Figure 1 shows the differential cross sections for ω production as a function of $\sqrt{s}$ [1-3]. Each panel shows the results in the ω scattering angle regions. In $-0.9 < \cos\theta_{\text{CM}} < -0.8$, the results of CLAS are consistent with the present results. The previous results of LEPS are also consistent with the present results. The differential cross section decreases as $\sqrt{s}$ increases above $\sqrt{s} \sim 2.3$ GeV in all the scattering angle bins. The smooth lines in Fig. 1 represent the theoretical calculations, which do not include the contribution of nucleon resonances. The LEPS results show that the present $\sqrt{s}$ dependence of the differential cross section differs from the theoretical calculation implying that the present $\sqrt{s}$ dependence is difficult to explain by only the baryon exchange process, and that there is a significant contribution from nucleon resonances in this energy range.

A possible candidate with high angular momentum is $G_{17}(2190)$. The coupling of the $G_{17}(2190)$ to $p\omega$ decay is supported by the analysis of the CLAS results [2]. The difference between the present data and the theoretical curve can be interpreted as an influence of $G_{17}(2190)$.

Figure 2 shows the differential cross sections for

η' production as a function of $\sqrt{s}$ [1,4,5]. Each panel shows the results at the η' scattering angle regions. The results of CB-ELSA in $-1.0 < \cos\theta_{\text{CM}} < -0.8$ are also shown in Fig. 2. The bump structure at $\sqrt{s} \sim 2.35$ GeV appears clearer in the most backward scattering angle bins, $-1.0 < \cos\theta_{\text{CM}} < -0.90$. Theoretical calculation is also shown in Fig. 2. In the calculation, various parameters such as the resonance mass and width in this calculation are tuned to reproduce the various experimental results [4]. The dotted lines in Fig. 2 show the baryon exchange contribution in the theoretical calculation [4]. It should be mentioned that the theoretical baryon exchange contribution at $\sqrt{s} > 2.35$ GeV is just an extrapolation. The dominant contribution at backward angles is from nucleon resonances at $\sqrt{s} < 2.10$ GeV and the $\sqrt{s} \sim 2.35$ GeV region according to the comparison between the LEPS results and the theoretical baryon exchange contribution. Since the rapid change at only the backward angle may indicate a resonance with a high angular momentum, PWA, including the present results that show the bump structure, will be important information to be clear for the involving nuclear resonance.

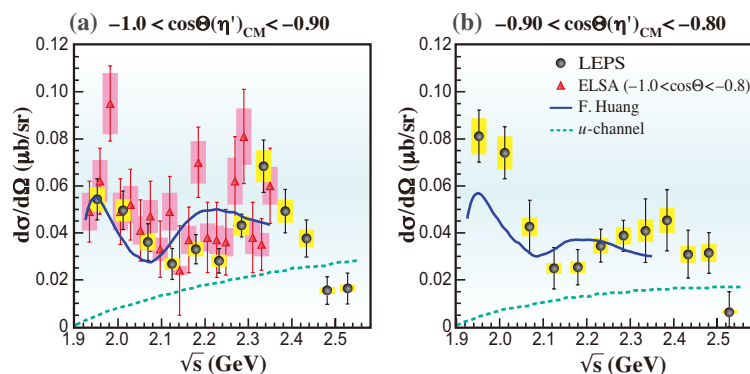


Fig. 2. Differential cross sections for η' photoproduction as a function of $\sqrt{s}$. Shaded bars represent the systematic uncertainty. Black circles are the present results [1]. Red triangles are the CB-ELSA results [5]. Smooth lines represent the theoretical calculation by F. Huang [4]. Dotted lines represent the u -channel contribution in F. Huang [4].

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References

- [1] Y. Morino, Y. Nakatsugawa, M. Yosoi, M. Niiyama, M. Sumihama, T. Nakano, D.S. Ahn, J.K. Ahn, S. Ajimura, W.C. Chang, J.Y. Chen, S. Daté, H. Fujimura, S. Fukui, K. Hicks, T. Hiraiwa, T. Hotta, S.H. Hwang, K. Imai, T. Ishikawa, Y. Kato, H. Kawai, M.J. Kim, H. Kohri, Y. Kon, P.J. Lin, K. Mase, Y. Maeda, M. Miyabe, N. Muramatsu, H. Noumi, Y. Ohashi, T. Ohta, M. Ōka, J.D. Parker, C. Rangacharyulus, S.Y. Ryu, T. Saito, T. Sawada, H. Shimizu, E.A. Stokovskiy, Y. Sugaya, K. Suzuki, K. Tanida, A. Tokiyasu, T. Tomioka, T. Tsunemi, M. Uchida, R. Yamamura and T. Yorita: *Prog. Theor. Exp. Phys.* (2015) 013D01.
- [2] M. Williams *et al.*: *Phys. Rev. C* **80** (2009) 065208.
- [3] A. Sibirtsev *et al.*: *nucl-th/0202083*.
- [4] F. Huang *et al.*: *Phys. Rev. C* **87** (2013) 054004.
- [5] V. Crede *et al.*: *Phys. Rev. C* **80** (2009) 055202.

ACCELERATORS & BEAMLINES FRONTIERS

SPRING-8 BEAM PERFORMANCE

Development and Upgrades of the Storage Ring

Improvement of Beam Lifetime by Adjusting Sextupole Magnets

At the present light source facilities, the top-up operation, where the electron beam is injected during user experiments to keep the stored current constant, is indispensable for precise experiments. Although the beam lifetime is no longer a critical issue for the present light sources due to the top-up operation, it is still important for radiation safety to extend the beam lifetime or to suppress the beam loss. The Touschek effect, which is the beam loss driven by the momentum change through the collision in the electron bunch, dominates the beam lifetime in the high brilliance light source ring. Since a scattered electron with large momentum deviation at a dispersive section starts to oscillate transversely, the momentum acceptance is restricted by the dynamic or physical acceptance as well as the RF acceptance [1-4]. To prevent the insertion devices, especially the in-vacuum undulators, from demagnetization, the reduction of the electron loss is crucial. Furthermore, when the top-up operation is halted due to a machine trouble in the injection, the long beam lifetime is effective in suppressing the rapid decrease of the stored current.

In May 2013, the emittance of the SPRING-8 storage ring was improved from 3.5 to 2.4 nm·rad by changing the ring optics. At the same time, the momentum acceptance decreased from 3.2 to 2.4%, which led to the reduction in the Touschek beam lifetime by half. Figure 1 shows the measured Touschek lifetime as a function of the RF accelerating voltage. The dashed line represents the estimated Touschek lifetime under the assumption that the lifetime is limited only by the longitudinal momentum acceptance, i.e., the RF bucket height. For easy comparison of the momentum acceptance, the lifetime is normalized by the bunch volume. The measured lifetime is saturated above some RF accelerating voltage where the transverse dynamics rather than the RF bucket height determines the lifetime. In order to enlarge the momentum acceptance, we optimized the dispersion and the dynamic aperture by using sextupole magnets. As a result, the Touschek lifetime has been improved up to the previous level with larger emittance as shown in Fig. 1.

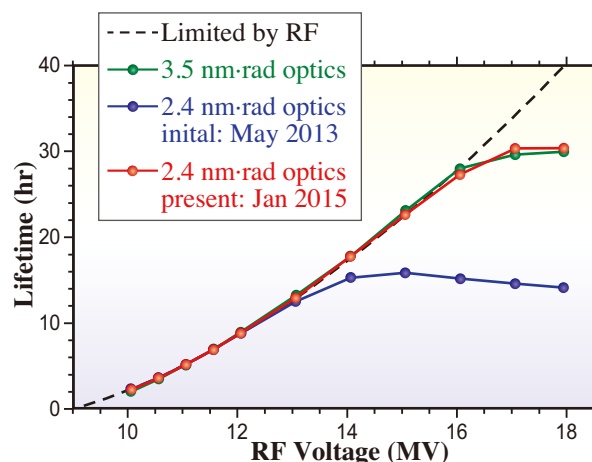


Fig. 1. Touschek lifetime as a function of RF accelerating voltage.

Development of a Ceramic Chamber Integrated with Pulsed Magnet

We are pushing forward the development of an air-core coil pulsed magnet that has an integrated structure of magnet coils with a ceramic vacuum chamber, aiming to reduce the gap size.

With a small gap, we can increase the magnetic field on the beam orbit. As a result of the increased magnetic field, the magnet length becomes shorter while keeping the kick angle the same. A narrow gap magnet reduces the coil inductance and, hence, the pulsed power supply load. As a result, a short pulse width with a high-repetition pulse is much more easily achievable while realizing the necessary kick angle.

In the structure we are developing, single-turn air-core coils are implanted along the longitudinal axis in the cylindrical 5-mm-thick ceramic chamber wall.

There are two technical advantages of applying this structure. The first is that the all-in-one structure makes the system simpler; three functionalities are included by implanting the coil in the ceramic chamber wall: (i) vacuum separation, (ii) the coils are held mechanically, and (iii) electrical insulation of the coils from the vacuum chamber. With this structure, magnet pole edges can be set close to the inner diameter of the chamber. Looking from the inside of the ceramic chamber, the pole edges are placed on the inside surface of the chamber and not bulge inward from the inner surface. Therefore, the pole edges are located close to the beam without disturbing the beam impedance.

The second is that the air-core coils are arranged around the circle on the inside surface of the ceramic to optimize the magnetic field strength and uniformity. There is no structural restriction in the arrangements of the coils or any complex coil-supporting structure. As a whole, a pulsed magnet will be built with an extremely simple component that does not bulge on the inside or toward the outside of the ceramic chamber.

The key technology in this magnet development is to implant the coil into the ceramic with a varying thermal expansion rate along the longitudinal axis. The development started with an implanting technique for the magnet coil in the ceramic chamber wall in 2012. A dipole-type prototype pulsed magnet was successfully manufactured with a bore radius of 30 mm and a magnetic length of 0.3 m in 2013 (Fig. 2). Figure 3 shows the aging setup with the vacuum pump and pulsed power supply. Continuous operation over 200 days from 2013 to 2014 was achieved without any failure under the condition of current excitation with a 20 kV/7.7 kA pulse with a 4 μ sec width and a repetition of 1 Hz in a thermal cycle ranging from 30°C to 80°C, by using the dipole prototype, while maintaining the vacuum degree better than 10^{-6} Pa. The measured magnetic field strength and uniformity agreed with calculation results within 1%.

We are continuing the development to make the magnet installable in a storage ring as an accelerator device as the next step. The problem to be solved for the next step is to develop an inner coating to enable the flow of the wall current induced by the electron beam, which is necessary for matching the chamber impedance in order to avoid instabilities. The coating of the inside surface of the ceramic chamber should be the 2–3- μ m-thick metal layer around the coil while maintaining the electrical insulation between the coils and metal layer. This technique will be expanded to multipole pulsed magnets, such as quadrupole or sextupole pulsed magnets and/or small radius pulsed magnets whose radius is 10 mm or smaller.

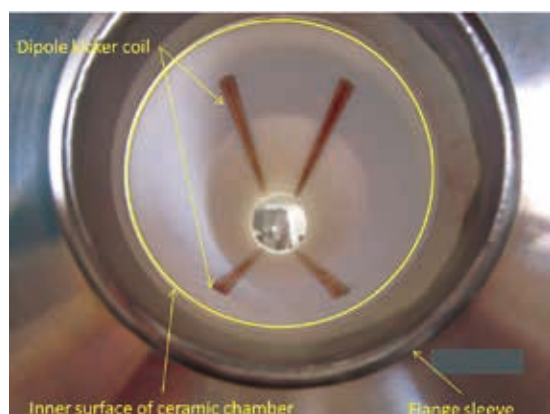


Fig. 2. Inside surface of the ceramic chamber in the dipole-type prototype magnet.

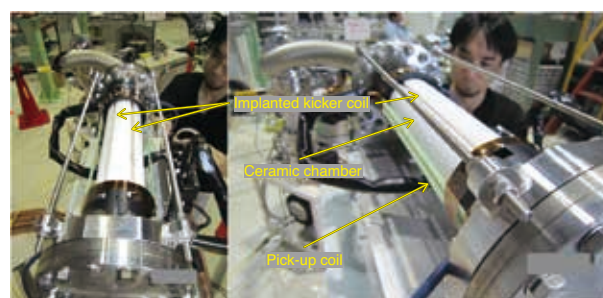


Fig. 3. Aging setup with vacuum pump and pulsed power supply.

New Magnet Alignment System Using Laser and Iris Diaphragms

The magnets on the common girders of the storage ring have a very tight alignment tolerance. For SPring-8, it is 50 μ m in the beam transverse directions, while for SPring-8-II, which is at the planning stage, it will be 25 μ m. To align these magnets, we have used a laser CCD-camera system and obtained reasonably good alignment results. The only shortcoming of the old system is the time-consuming measurement because great care must be taken when setting the camera's position to obtain good reproducibility at each measurement point. This alignment system should be replaced with a new one capable of high precision and high speed.

A new system that uses a laser and irises has been developed as shown schematically in Fig. 4. The system utilizes the Fraunhofer diffraction of a circular aperture (iris), known as an Airy pattern, and measures the distance between the centers of iris images to determine the distance between two irises.

The new laser alignment system is considered to solve the problem of camera setting and uses iris diaphragms as the targets [5]. At the point of measurement, the diaphragm closes to its minimum caliber, and then opens to its maximum caliber after measurement to let the laser beam pass.

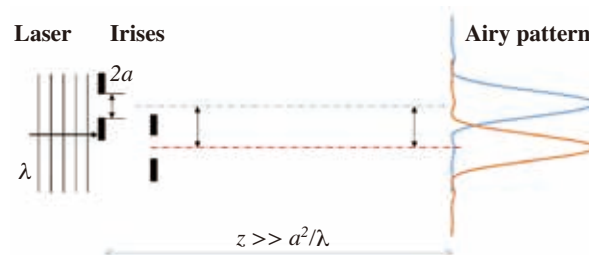


Fig. 4. Principle of the laser and iris alignment system.

We previously attempted a laser-iris system in 2006, as part of the SCSS (SPRING-8 Compact SASE Source) project. In the previous system, a laser beam was introduced into the vacuum chamber. Retractable irises, having fixed apertures from 2 to 5 mm, were inserted into the chamber with compressed air cylinders and were used to measure the positions of the BPMs in sequence along the beamline. For this system, it is important to have good reproducibility of the iris center. Although the initial reproducibility achieved was better than $1\text{ }\mu\text{m}$, the reproducibility gradually became worse due to friction and impact between the iris and BPM bodies.

The new system for the storage ring uses iris diaphragms as the targets. Commercial iris diaphragms were tested but did not meet the precision requirement. They usually have central reproducibility of a few micrometers, which is insufficient for our requirement. Therefore, we have developed a new diaphragm (Fig. 5). This device consists of 11 blades, a base plate, centering balls and an actuating ring driven with a gear. The rotation parts are very precise and smooth. The device has variable calibers from 1-12 mm ϕ . The reproducibility of the diaphragm center was measured to be under $\pm 1\text{ }\mu\text{m}$ in 100 trials. The iris diaphragms are used at 1-2 mm ϕ calibers depending on the distance from the camera and are opened and closed with a remote controller.

The new laser alignment system is composed of a laser source, a CCD camera and four diaphragm targets (Fig. 6). The two end diaphragms are set on the reference points of a girder, and the two inner diaphragms are placed on the magnets in measurement. The total length of the design is 10 m.

The laser head consists of a 3.5 mW fiber-coupled diode laser of 0.635 μm wavelength, a collimator and a 6 $\times$ beam expander. The output laser beam has a 5 mm diameter, which is adjusted to cover a 10 m measurement range. For an alignment

tool, pointing stability of the laser is essential. The pointing stability was tested and found to be under $\pm 2\text{ }\mu\text{m}$ (σ) in 8 m when the cooling water and air conditioner were stopped.

A Redlake Megaplus II ES4020 CCD camera is used. It has a small pixel size of $7.4 \times 7.4\text{ }\mu\text{m}^2$ and a large sensor size of $15 \times 15\text{ mm}^2$ with 2048×2048 pixel resolution. Images of 8 Mb are acquired at a frame rate of 15 fps through a CameraLink interface. For each frame, pixel values are summed in rows or columns to obtain one-dimensional intensity distributions of x and y. The centroid of an Airy disk is calculated at a clip level of above 13.5%. The camera system was verified to have a resolution better than $1\text{ }\mu\text{m}$ using a precise automatic stage.

The performance of the new laser alignment system was examined by 10 repeated measurements of the magnet positions on a common girder. It was verified that the system has a measurement uncertainty of $\pm 10\text{ }\mu\text{m}$ (2σ) in a 8 m range, which even meets the requirement for the future accelerator of SPRING-8-II.

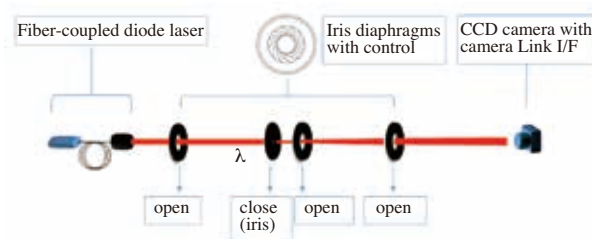


Fig. 6. Schematic of the iris diaphragm laser alignment system.

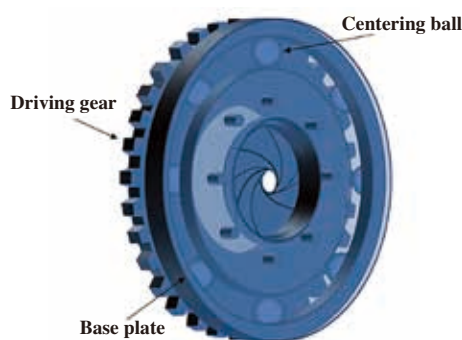


Fig. 5. Schematic of developed iris diaphragm.

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References

- [1] M. Takao *et al.*: Proc. EPAC00 (2000) p. 1569.
- [2] M. Takao *et al.*: Proc. 13th Symposium on Accelerator Science and Technology (2001) p. 316.
- [3] H. Tanaka *et al.*: Proc. EPAC02 (2002) p. 1305.
- [4] M. Takao *et al.*: Proc. 3rd Annual Meeting of Particle Accelerator Society of Japan and 31st Linear Accelerator Meeting in Japan (2006), 898 (FP53).
- [5] C. Zhang and S. Matsui: Proc. IWAA2012, Fermilab, USA (2012).

Major upgrade of BL25SU for soft X-ray imaging and spectroscopy using nano- and micro-focused beams

Here we report a recent major upgrade of beamline **BL25SU** for soft X-ray spectroscopy of solid. The upgrade project started in 2012 for the purpose to optimize the beamline optics and experimental stations for advanced soft X-ray imaging and spectroscopy using nano- and micro-focused beams. Particularly, magnetic imaging with soft X-ray magnetic circular dichroism (XMCD) using a nano-focused beam ($<\phi 100$ nm) is highly required in accordance with the element strategy project of permanent magnets, initiated by Ministry of Education, Culture, Sports, Science & Technology in Japan.

The original beamline was one of the first ten beamlines constructed at SPring-8 in 1998 [1]. BL25SU has mainly been dedicated to soft X-ray photoemission spectroscopy (PES) and absorption spectroscopy for studies on the electronic and magnetic properties of solid-state materials. A helicity switching technique using a twin helical undulator light source [2] has been advantageous when measuring soft XMCD precisely. To promote high-energy-resolution PES, the beamline was designed to achieve high energy resolution of about $E/\Delta E > 10,000$ at a photon energy of 1 keV.

During reconstruction, every optical component at the original beamline was replaced with new components. Installation of the new beamline started in Jan. 2014, and was completed in three months. After the half-year commissioning during the 2014A experimental period, the beamline has been available to users since 2014B. The new beamline consists of two branches: a microbeam with high-energy-resolution branch (branch-a) and a nanobeam with small angular divergence branch (branch-b). These can be switched using the pre-focusing mirrors (M0) shown in Fig. 1. Downstream of M0 for vertical focusing, each branch has its own horizontal focusing mirror (M1). A monochromator based on the Hettrick Underwood type [3] is composed of an entrance slit (S1), spherical mirrors (M2n), varied line spacing plane gratings (Gn), and an exit slit (S2). The specifications of each branch are listed in Table 1.

The beam performance of branch-a is almost the same as the original one in terms of its energy resolution, energy range, and photon flux. This branch has three experimental stations, ST1a: two-dimensional photoelectron spectroscopy

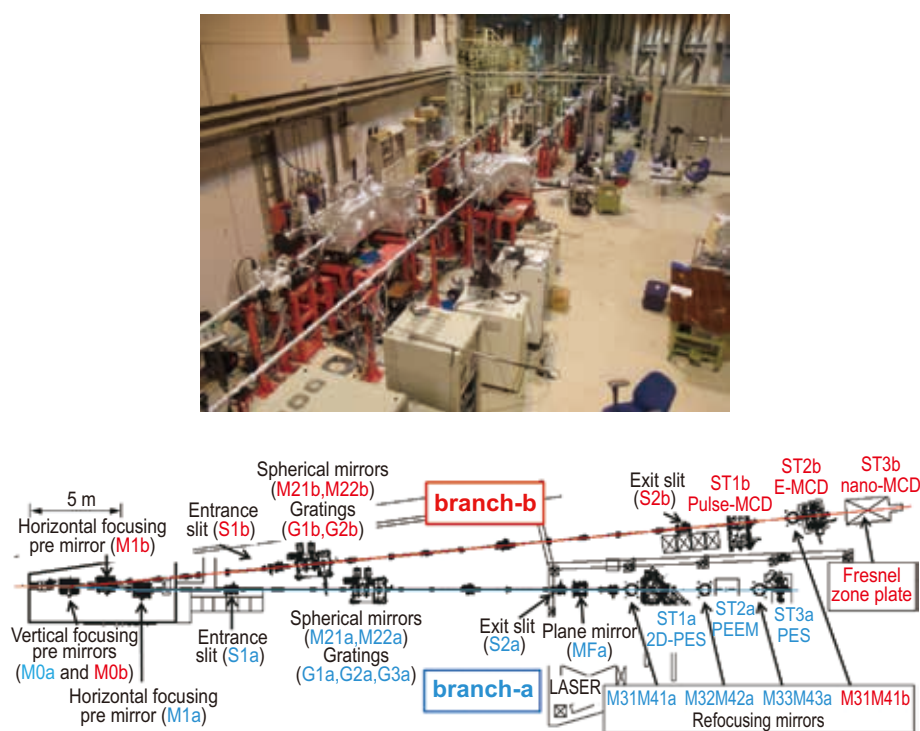


Fig. 1. Photograph and schematic view of upgraded BL25SU.

Table 1. Design specifications of each branch of upgraded BL25SU.

	Branch-a High energy resolution	Branch-b Small angular divergence
Energy range	120 – 2000 eV	200 – 2000 eV
Resolving power	$E/\Delta E > 10,000$	$E/\Delta E > 3,000$
Photon flux	10^{11} ph/sec	10^{12} ph/sec 10^{10} ph/sec @ nano-MCD
Beam Size	10 – 100 μm	10 – 100 μm $\phi 100$ nm @ nano-MCD

(2D-PES) apparatus, ST2a: photoemission electron microscope (PEEM), and ST3a: photoemission electron spectroscopy (PES) apparatus. In order to obtain a micro-focused beam, refocusing mirror systems (M3n, M4na) are installed upstream of each experimental station. A high resolving power ($E/\Delta E$) of above 10,000 is confirmed by the photoemission profile at the Fermi edge of Au. The photon flux

of the monochromatized beam is measured to be 10^{11} photons/sec/0.01%BW using a Si photodiode. The focused beam sizes at three end stations are typically better than 10 μm (V) $\times$ 170 μm (H).

The optical layout of branch-b is optimized for nanofocusing using a Fresnel zone plate (FZP). To increase the acceptance of FZP, the divergence angle after the exit slit is suppressed by the asymmetrical layout of the monochromator components. The divergence in a vertical direction at branch-b is about 1/10 of that at branch-a, as shown in Fig. 2. There are three experimental stations, ST1b: pulse-magnet-type XMCD apparatus (Pulse-MCD), ST2b: electromagnet-type XMCD apparatus (E-MCD), and ST3b: XMCD apparatus equipped with the FZP (nano-MCD). A refocusing mirror system (M31, M41b) is installed upstream of E-MCD. The resolving power and photon flux after the exit slit are 4,000 and 6×10^{11} photons/sec/0.03%BW, respectively. The estimated focused beam sizes are 10 μm (V) $\times$ 100 μm (H) at E-MCD and ϕ 100 nm at nano-MCD.

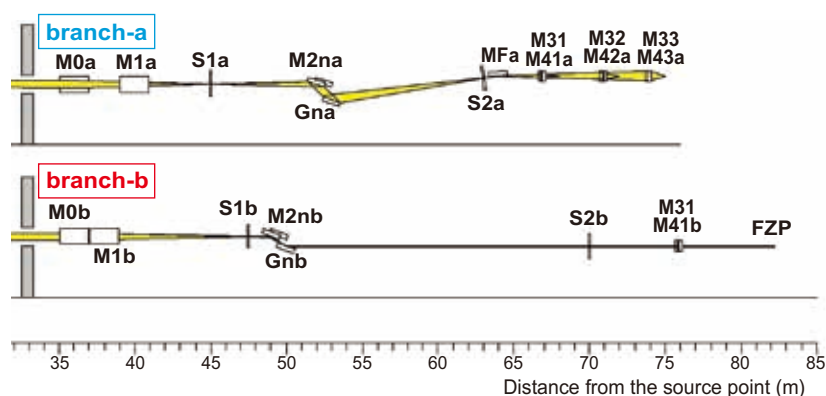


Fig. 2. Optical layout (side view) of upgraded BL25SU.

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References

- [1] Y. Saitoh *et al.*: J. Synchrotron Rad. **5** (1998) 542.
- [2] T. Hara *et al.*: J. Synchrotron Rad. **5** (1998) 426.
- [3] M.C. Hettrick and J.H. Underwood: AIP Conf. Proc. **147** (1986) 237.

Development of high lateral and wide angle resolved hard X-ray photoemission spectroscopy

Hard X-ray photoemission spectroscopy (HAXPES) has become a powerful tool to investigate the chemical states and electronic structures of various materials using third generation high-brilliance synchrotron radiation sources. The most advantageous feature of HAXPES is that it enables intrinsically bulk sensitive electronic structures and buried interface profiles to be measured. The variable depth analysis of electronic properties by HAXPES has realized various applications both in basic and applied research fields [1]. Such research is targeting nanoscale-microscale materials, leading to more requests for observations in this area. Hence, it is important to investigate the electronic states, including edge structures and cross sections with depth profiles, over the nanometer to micron scale. Anticipating demands from rapidly developing nanoscale- microscale science and technology, the 2D scanning of a sample with finer micro-focused X-rays is necessary to obtain a higher lateral resolution. We have targeted the 3D analysis of chemical states by utilizing the large probing depth of HAXPES together with the variable takeoff angles provided by a wide-angle lens. Our development approach on the HAXPES scanning microscope combines the Kirkpatrick-Baez focusing mirrors (K-B) and a wide-acceptance-angle electrostatic lens (WAAELs) with angular resolving capability at beamline BL47XU [2].

By installing the K-B mirrors, we achieved a beam size of $1.10 \text{ (H)} \times 0.98 \text{ (V)} \mu\text{m}^2$ at photon energy of 7.94 keV as a lateral resolution. Both the vertical and horizontal focusing mirrors are elliptical, and the lengths of both mirrors are 100 mm. The working distance of the K-B system is 350 mm. The magnification is $0.35/50=1/143$ to locate K-B system at a position 50 m from center of the undulator. In other words, the divergence is 143 times. Development of a WAAEL with an angular resolution capability is also necessary to realize 3D chemical analysis via angle-resolved X-ray photoelectron spectroscopy. Details of the simulation results for this WAAEL are described elsewhere [2,3]. The total magnification factor increases 25 times by combining the objective and the analyzer lenses. This means that the effective view field on the sample with the entire analyzer system is 5 times smaller compared to the analyzer without a WAAEL. The efficiency of the electron detection thus increases while

decreasing the X-ray spot size on the sample surface. Thus, the combination of a WAAEL and the $1\text{-}\mu\text{m}^2$ focusing of the K-B mirror effectively increases the throughput of the photoelectron measurements. The result of the evaluation of acceptance angle is shown in Fig. 1. Figure 1(a) shows the 2D image record of the Au valence band spectrum near the Fermi edge region in the angular mode. The number of slice peaks can be directly indicated by acceptance angles larger than $\pm 32^\circ$, because the period of the multi-slit is 2.8° . The magnified profile of one slice is shown by red circles in Fig. 1(b). The blue curve in Fig. 1(b) is the derivative of the edge shape of the slice profile. The angular resolution is estimated to be 1.32° from the FWHM of the derivative. The spherical aberration is apparent in high emission angles (Fig. 1(c)). The aberration of 22 meV at the maximum is remarkably small for the total energy resolution of 265 meV. It is thought that this aberration originates in the accuracy of an ellipsoidal mesh, in which the gap from a mesh-shaped design is currently about $50 \mu\text{m}$. The high-precision fabrication of an ellipsoidal mesh is the most important point, and much research and development was conducted to remove this aberration.

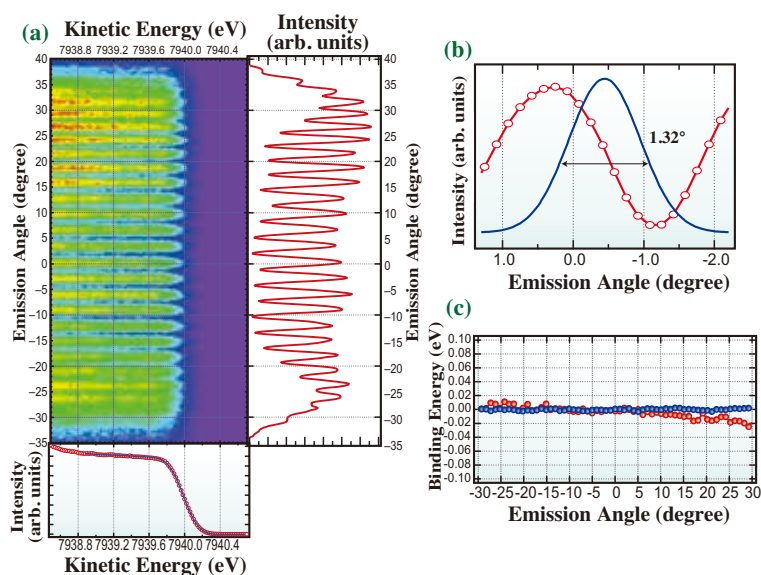


Fig. 1. Test results for the angular acceptance and resolution of the WAAELs obtained using a test device composed of a Au plate, in front of which a combination of a cylindrical multi-slit (the period of the multi-slice is 2.8°) was mounted. (a) 2D image recorded in the Au Ef region in angular mode. Right and bottom show the integrated profiles of the slit image along with the kinetic energy and the Au Ef spectrum along with emission angle, respectively. (b) Magnified profile of one slice (red circles) and the differential curve in the edge shape of this slice profile (blue line). (c) Raw plot of Fermi level of Au for binding energy at each acceptance angle (red circles) and after correcting for energy (blue circles).

Here, some of the results that combine K-B mirrors with a WAAEL are described. All the experiments were measured at a photon energy of 7.94 keV. The performance of this scanning depth analysis was evaluated by carrying out core spectra measurements of a typical multilayered sample of Ir (8 nm)/HfO₂ (2.2 nm)/ thickness-graded (TG) SiO₂ (0–10 nm)/Si (001), schematically shown in Fig. 2(a). By scanning the position of the TG sample surface along the SiO₂ thickness and varying the direction stepwise in 50- μ m increments, the Si 1s and O 1s spectra are found to exhibit the features shown in Figs. 2(b) and 2(c), respectively. The Si 1s from the SiO₂ layer loses intensity and shifts towards a lower binding energy as the thickness decreases. In addition, the depth profile of the structure has been mapped along the SiO₂ thickness varying direction with the TOA dependence of these core spectral intensities. These results directly indicate that band bending depends on thickness due to the charge trap of interlayer band diagram [4]. This charge trap mechanism proposes that positive charges are trapped on the HfO₂ surface, while negative charges are trapped in the HfO₂ film along thickness of SiO₂.

Furthermore, the scanning and angle-resolved results of 2D mapping applied to micro-dots of (Fe_{2.5}Mn_{0.5})O₄ ferromagnetic oxide (thickness: 20 nm) on Nb_{0.5}wt%-SrTiO₃ substrates are shown in Fig. 3. This (Fe_{2.5}Mn_{0.5})O₄ system is a typical magnetic semiconductor exhibiting strong electron correlation effects [5]. The valence band spectra revealed that the DOS near E_F of (Fe_{2.5}Mn_{0.5})O₄ thin films systematically decreased as the Mn concentration increased. The band shift due to changing the electron correlation was observed in a wide substitution range and is interpreted

by the change on the basis of the Mott-Hubbard model [5]. The dot size is 50 $\times$ 50 μ m² in the optical image of Fig. 3(a). In Figs. 3(b) and 3(c), the 2D mapping image of integral intensity for the Fe 2p_{3/2} and Sr 3p_{3/2} shows mapped with 1- μ m steps, respectively. The micro-dot is well resolved using a 1- μ m beam size. This lateral-resolved investigation of a micro-dot thin film suggests that this material system has potential to develop advanced spin electronics. Through a combination of the K-B mirrors and the WAAEL, chemical state mapping will provide important information about the electronic properties of various materials crucial to basic science, industry and technology.

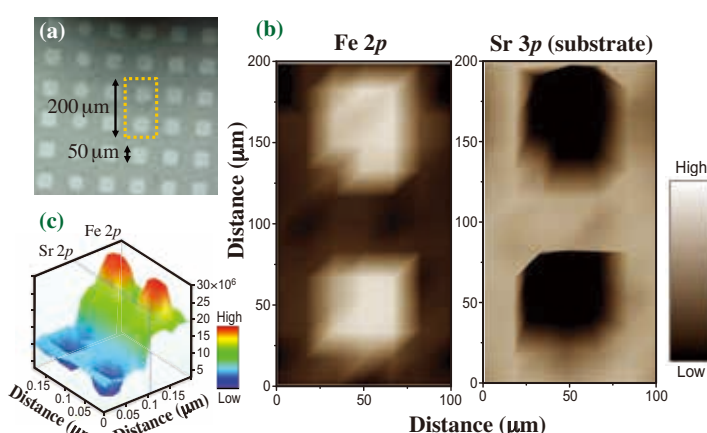


Fig. 3. Results of 2D mapping via sample scanning applied to a rectangular micro-dot of (Fe_{2.5}Mn_{0.5})O₄ ferromagnetic oxide (thickness, 20 nm) on a Nb_{0.5}wt%-SrTiO₃ substrate. (a) Dot size is 50 $\times$ 50 μ m² in the optical image. (b) and (c) 2D mapping image of the integral intensity for Fe 2p_{3/2} and Sr 3p_{3/2}, respectively.

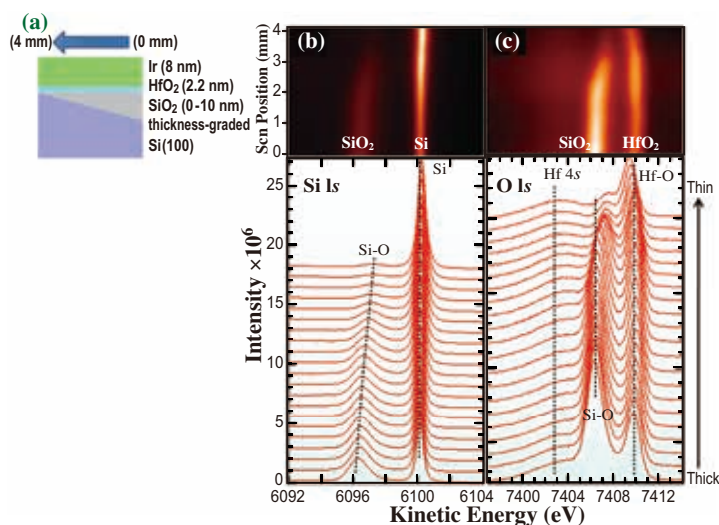


Fig. 2. (a) Schematic structure of the Ir (8 nm)/HfO₂ (2.2 nm)/SiO₂ (0–10 nm)/Si multi-layer. (b) and (c) Position dependence of Si 1s and O 1s along the TG direction by increments of 50 μ m, respectively.

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References

- [1] K. Nomura *et al.*: Nature **432** (2004) 488; K. Nomura *et al.*: J. Appl. Phys. **109** (2011) 073726.
- [2] E. Ikenaga, M. Kobata, H. Matsuda, T. Sugiyama, H. Daimon, K. Kobayashi: J. Electron Spectrosc. Relat. Phenom. **190** (2013) 180.
- [3] H. Matsuda *et al.*: Phys. Rev. E. **71** (2005) 066503.
- [4] Y. Abe *et al.*: Jpn. J. Appl. Phys. **48** (2009) 041201.
- [5] J. Takaobushi *et al.*: Phys. Rev. B **76** (2007) 205108.

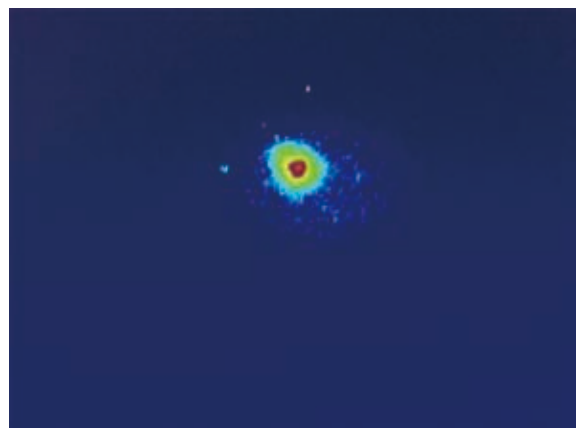
SACLA BEAM PERFORMANCE

SACLA provided routine operations for users in 2014, despite an accident in the injector system in May 2014 that caused some laser instabilities. This problem was successfully resolved in January 2015. The construction of BL2, a new XFEL beamline, was completed on schedule during our two-month summer shutdown. Although the relatively long summer shutdown decreased the total operation time by 10%, the total public user time increased by 100 hours due to improvements in the efficiency of the tuning and preparation procedures.

Successful beam commissioning of our new beamline, BL2

The construction of BL2, our newest beamline, was completed during the summer shutdown. [Figure 1](#) shows BL2's undulator line, which is composed of 15 undulator segments. Beam tuning began in the last week of September and the facility passed its inspection on October 7. The first laser amplification at BL2 was achieved on October 21 and the laser intensity reached around 20 μJ per

pulse at 10 keV. [Figure 2](#) shows the laser profile measured in the optical hutch. During the winter shutdown, two inverse bending magnets were installed in the beam transport line to enable precise beam tuning to achieve a higher laser intensity. The DC beam-switcher was replaced by a combination of a pulse-kicker and DC septum magnets in order to achieve pulse-by-pulse laser switching operations.



[Fig. 2.](#) First lasing achieved at the BL2 undulator line.



[Fig. 1.](#) New BL2 undulator line (left) installed in the SACLA undulator hall together with the existing BL3 (right).

Accelerator stabilization and operation reliability improvement

One remaining instability issue is not shot-by-shot fluctuation but a slow parameter drift. We thought that timing drift might be the primary cause of this parameter drift. Therefore, we built an optical-fiber length stabilization system for a reference RF signal transmitter. The system employs an optical interferometer to measure the optical path-length variation and a piezoelectric fiber stretcher to compensate for the variation. By using this system, the timing drift of the reference RF signal transferred through an optical fiber several hundred meters long is suppressed to within 50 fs [1].

One of the critical issues for reliable SACLA operation is to reduce the downtime caused by

breakdowns of the thyratrons (the high-voltage plasma switches). SACLA uses more than 70 thyratrons and after three years in operation, most of them are approaching the end of their estimated service life. We have made extensive efforts to shorten the downtime and to ensure a sufficient number of backup thyratrons [2]. For example, we modified a thyatron protection circuit so that the TVS (transient voltage suppressor) diodes are located outside the insulation oil tank, because TVS diodes frequently break down after high-voltage surges as thyratrons age. This modification simplifies repairs and has greatly contributed to making operations more reliable.

Development of a self-seeded XFEL at BL3

We developed an XFEL self-seeding system using a Bragg transmission scheme at BL3 of SACLA to generate a single-mode-like laser with a sharp and constant spectrum. The setup is composed of a small magnetic chicane, which can delay an electron beam of 8 GeV by 50 fs at maximum and a diamond single crystal with a thickness of 180 μm .

Although beam commissioning for the self-seeding system remains underway, we were able to achieve four times higher intensity and a ten times narrower spectral bandwidth at 10 keV compared with those of SASE as shown in Fig. 3 [3]. Further accelerator tuning will continue to improve the reproducibility and long-term stability of the self-seeded XFEL.

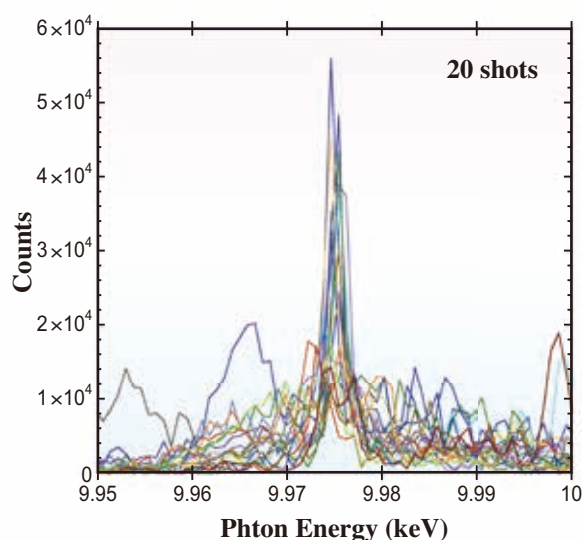


Fig. 3. Single-shot spectra of self-seeded XFELs.

Electron beam instability in the injector

Early on the morning of May 26th the mis-steering of 500 keV electron beams during successive measurements of current setting errors for all the electromagnets broke a bellows at the injector and caused a vacuum leak there. The chamber component with the broken bellows was quickly replaced with a similar one installed in the BL1 linear accelerator under construction and SACLA operations were restarted later that day. However, large fluctuations of the electron beam trajectories continued at the injector after the replacement, causing pointing instability for the XFEL and seriously restricting the XFEL performance.

The preliminary investigation of operations data suggested that a cause of the fluctuations may have been poor vacuum conditions at the gun cathode. To address this problem, during the summer shutdown we added an NEG pump system near the cathode. Unfortunately, the fluctuations were not suppressed at all. Further investigation revealed another potential cause - the build-up of charge at the ceramic chamber adjacent to the bellows by the electron bombardment kicked the beam at random intervals; we found that the replacement chamber component had a wider aperture than the original one, which resulted in increased charge build-up. During the winter shutdown, we exchanged the chamber component with a modified one with a halo scraper and a coated ceramic surface to suppress charge build-up. This modification finally succeeded in stopping the fluctuations.

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References

- [1] H. Maesaka *et al.*: Proc. IPAC2014, Dresden, Germany, (2014) p. 1906.
- [2] K. Masuda *et al.*: Proc. 11th Annual Meeting of Particle Accelerator Society of Japan, Aomori, Japan, (2014) p. 654.
- [3] T. Inagaki *et al.*: Proc. FEL2014, Basel, Switzerland, (2014) p. 603.

Extreme power density of hard X-ray free electron laser pulses generated by a two-staged reflective focusing system

The interactions between matter and light transform from linear to nonlinear phenomena as the intensity of light increases. The knowledge of nonlinear optics of visible light are well explored and commonly employed in various optical instruments and devices. Physicists are curious about X-ray nonlinear optics because photons with short wavelengths have substrate dependent responses. This phenomenon has been radical in astrophysics, fusion, and elementary physics. Due to the characteristic of permeability against matter and an insufficiently intensive X-ray source, only theoretical research has progressed. However, this situation has drastically changed from the viewpoints of X-ray sources and optical devices. SACLA produces the shortest-wavelength laser and reaches operations in the 0.6-Å wavelength range.

One of the major advantages of X-rays is the production of a nanometer-sized spot. Diffraction limit theory is a fundamental nature of light in which the wavelength determines the minimum resolution in imaging and focusing. In this decade, various X-ray focusing devices have advanced, reaching the diffraction limit performance. The focal size is continuously decreasing, and is as small as 10 nm [1]. Such focusing optics can enhance the characteristics of XFEL light by further increasing the power density, which is strongly desired to create extreme conditions of matter.

On the other hand, in other XFEL experiments, a focal length distance is necessary because the debris from targets due to radiation of intensive

XFEL pulses seriously degrades the optics performance and various equipment must be located around the sample. Although a large aperture is preferable, the extremely short-wavelength lasers propagate while expanding slightly because the expanding angle of the beam is proportional to the wavelength. A very long-beamline is necessary to obtain a large beam size. To overcome these fundamental problems with XFEL light, one more focusing system has been installed to expand the XFEL pulses. Furthermore, the focal beam size is drastically decreased by magnifying the XFEL light before the final focus.

A two-staged focusing system with two Kirkpatrick Baez geometries was designed, developed, and installed at beamline **BL3** of SACLA for focusing XFEL pulses to an extreme tiny size of about 50 nm (Fig. 1) [2]. Total reflection mirrors are employed to tune the wavelength in various analytical methods using XFEL pulses. The four precise mirrors were fabricated by a deterministic fabrication process, consisting of elastic emission machining and interferometric metrology. Figure accuracies are 2 nm (peak-to-valley) and surface roughness is 0.2 nm (root-mean-square). The first pair of focusing mirrors is 120 m downstream from the end of the undulator to focus XFEL pulses to several micrometers with nearly 100% efficiency [3]. Then XFEL pulses propagate with expansion until reaching the downstream focusing system. The second pair of focusing mirrors is located approximately 72 m downstream from the upstream device.

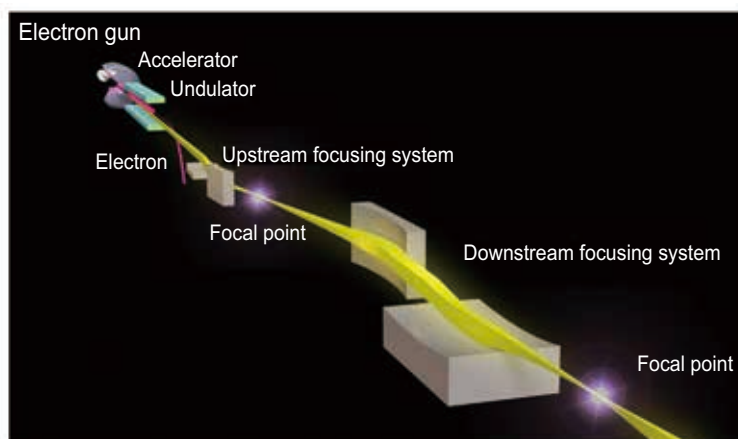


Fig. 1. Two stage reflective focusing system for XFEL focusing.

An XFEL focusing experiment was performed at 9.875 keV. At first, the upstream K-B system was aligned to measure the intensity profile of XFEL pulses at the designed focal point. After that, the downstream K-B system was adjusted to obtain the minimum spot size.

Figures 2 show the intensity profiles on the focal plane, which are estimated by a grating interferometer [4]. The focused beam sizes are 55 nm $\times$ 30 nm in the horizontal and vertical directions, respectively. The intensity profiles are almost the same as those under the diffraction limited conditions [2].

The power density is one of the crucial parameter of the focused XFEL pulse. The power of each XFEL pulse can be precisely measured at SACLA. In an experiment using a photon energy of 9.875 eV and a pulse duration of 7 fs, the average power at each pulse is confirmed to be 42 μ J/pulse at the downstream focusing system. In this experiment, at least 30% of the photons in the focused beam pass through a 50-nm square area on the focal plane. From the experimental outcomes, we can declare that the maximum power density exceeds at least 10^{20} W/cm².

Recently, XFEL has activated the X-ray nonlinear

optics field. At SACLA, this extremely strong X-ray laser has just opened the next phases for some experiments. For example, a nonlinear phenomenon called “saturable absorption of intense hard X-rays” has been released [5], which clearly shows a signal due to nonlinear interaction between X-rays and matter.

Now, not only XFEL facilities but also various types of next-generation synchrotron radiation facilities are being planned or constructed. These facilities will provide laser-like hard X-ray beams because high quality X-ray beams with extremely short wavelengths have a straight propagation. A two-staged focusing with long mirrors can control the beam size and allow freedom in the optical design, which will create unique X-ray experiment. In addition, single-nanometer XFEL focusing also requires this system. Thus, a power density of 10^{22} W/cm² has been achieved. The competition to realize extremely intensive photon beams will be more activate by the birth of XFEL nanobeam. It is believed that the two-staged focusing system will play a crucial role in driving X-ray lasers and lead to unprecedented outcomes in X-ray physics.

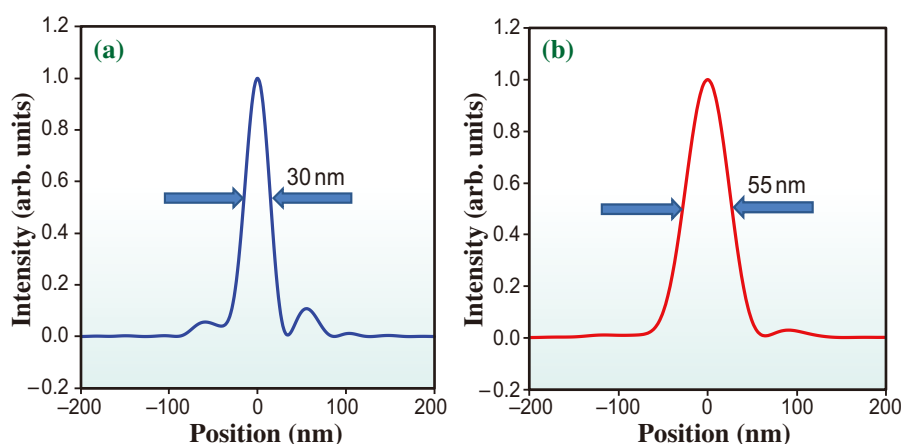


Fig. 2. Intensity profiles of XFEL focused beam in the (a) vertical direction (b) horizontal direction.

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References

- [1] H. Mimura *et al.*: Nat. Phys. **6** (2010) 122.
- [2] H. Mimura, H. Yumoto, S. Matsuyama, T. Koyama, K. Tono, Y. Inubushi, T. Togashi, T. Sato, J. Kim, R. Fukui, Y. Sano, M. Yabashi, H. Ohashi, T. Ishikawa and K. Yamauchi: Nat. Commun. **5** (2014) 3539.
- [3] H. Yumoto *et al.*: Nat. Photonics **7** (2013) 43.
- [4] S. Matsuyama *et al.*: Opt. Exp. **20** (2012) 24977.
- [5] H. Yoneda *et al.*: Nat. Commun. **5** (2014) 5080.

Development of a diamond X-ray phase retarder to generate a circularly polarized XFEL beam at SACLA

An X-ray free electron laser (XFEL) is a light source that combines the unique features of laser and X-rays. XFEL radiation is characterized by extremely high brilliant, transversely coherent, and ultrashort X-ray pulses. Using this probe with unprecedented capabilities, atomic-scale observation of ultrafast dynamics in materials and biological systems would be feasible. XFEL could offer potential applications that have yet to be explored using storage-ring-based synchrotron radiation. One unexploited feature of XFEL is X-ray polarization. This is in contrast to variable X-ray polarization, which has been a standard technique used in various applications at storage ring sources.

Generally, XFEL sources produce a linearly polarized electromagnetic wave, in which the electric field vector oscillates in the horizontal plane. However, researchers have desired XFEL beams in other polarization states where the electric field vector is rotating spirally (i.e., circular polarization). Circularly polarized XFEL can extend the range of SACLA's applications, making it possible to observe electron spin motions in magnetic materials and structural dynamics of chiral materials in biological systems in a time scale shorter than 1 picosecond. Full utilization of the XFEL polarization property should expand the capability of this powerful light source.

In this study, we developed an instrument to control the polarization states of an XFEL beam [1]. **Figure 1** shows a schematic of our X-ray polarizer optics, which is similar to the system established at the beamlines of storage ring sources [2]. A synthetic diamond crystal was used as a transmission-type phase retarder. The diffraction plane is tilted 45° from the direction of incident linear polarization, and the crystal angle is set at near the Bragg condition. As X-rays pass through the crystal, a phase shift between the σ - and π -polarization components is generated, which is controllable with the crystal angle. At an optimum angle, which gives a $\pi/2$ -phase retardation, the diamond crystal works as a quarter-wave plate and it converts the linearly polarized XFEL beam generated from SACLA into circular polarization.

We made a dedicated vacuum chamber for the diamond phase retarder, shown in **Fig. 2**. The chamber was installed in the optics hutch at beamline **BL3** of SACLA. A diamond crystal is placed at the center of the chamber and can be rotated using the ω -2 θ goniometer. Three diamond crystals with different thicknesses (0.1, 0.5, and 1.5 mm) are mounted on a

crystal holder and can be selected without breaking the vacuum. These crystal thicknesses were designed to cover an X-ray energy range from 5 to 16 keV. The chamber is a standard optical component of the beamline, which is installed permanently and available for routine use in XFEL experiments that require variable polarization states.

In an X-ray polarization control experiment, a (100)-oriented diamond crystal of 1.5 mm thickness was used in the transmission Laue geometry of the 220 reflection. We monitored the X-ray magnetic circular dichroism (XMCD) signals from a ferromagnetic sample to determine the degree of circular polarization. XMCD is defined by the difference in the X-ray absorption of a sample between right- and left-handed circular polarization. The intensity of XMCD is proportional to the degree of circular polarization (P_C). We used a powder sample of a CoPt₃ alloy, which was magnetized by an electromagnet. An XFEL beam transmitted through the diamond phase retarder was introduced to the sample at an X-ray energy of the Pt L_3 edge, 11.562 keV.

Figure 3 shows the measured variation of the XMCD signal as a function of the angle of the diamond crystal ($\Delta\theta$). The origin of the horizontal axis corresponds to the exact Bragg angle for the 220 reflection. For comparison, the solid line denotes the P_C values calculated by dynamical theory. Left-handed circular polarization with a nearly perfect degree of $P_C = -0.97$ was obtained at $\Delta\theta = 31$ arcsec. The helicity was reversed at a negative offset angle, and right-handed polarization was generated at $\Delta\theta = -31$ arcsec, but the degree of circular polarization was only $P_C = 0.82$.

The measured variation of the polarization state was in good agreement with the calculation for a positive angular range. P_C for a negative offset was

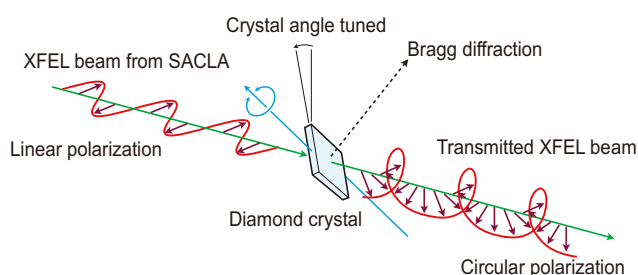


Fig. 1. Schematic of the X-ray polarization control optics using a diamond phase retarder.

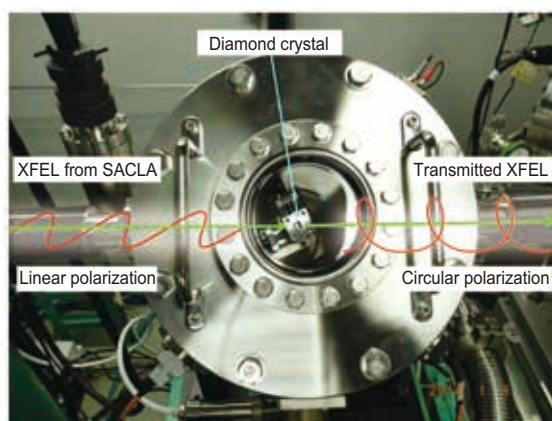


Fig. 2. Vacuum chamber dedicated to the diamond phase retarder, installed at BL3 of SACLA.

lower than the values expected from the calculation. The purity of the circular polarization was probably degraded by the glitch observed at $\Delta\theta = -280$ arcsec (not shown). The P_C for a negative offset can be optimized by changing the azimuth of the diamond crystal by $1-2^\circ$ to remove the glitch. This result confirms that the helicity of circular polarization can be reversed by only a small change in the crystal angle (62 arcsec). Our system has a piezo-driven stage to quickly adjust the crystal angle, enabling angular oscillation at 100 Hz for sinusoidal and 40 Hz for rectangular motions. Helicity switching at these frequencies is feasible, enabling shot-to-shot polarization switching synchronizing with the XFEL pulses from SACLA.

In this study, we demonstrated that the diamond phase retarder works effectively for an XFEL beam to control the X-ray polarization states. Circularly polarized XFEL radiation with variable helicities has been successfully generated. The degree of circular polarization was sufficiently high for most polarization-dependent experiments. Our system has advantages in compactness, flexibility, and ability of fast polarization switching over the scheme of XFEL polarization control using specially-designed undulators. With the device currently installed, SACLA has become the only facility that can provide circularly polarized XFEL beams. This extended capability of SACLA will likely produce a number of leading-edge studies particularly in magnetic and chiral materials.

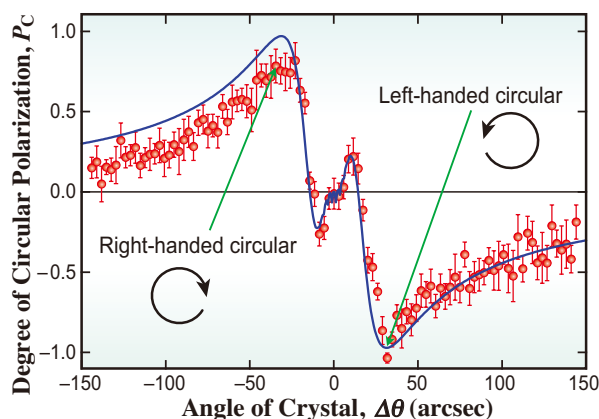


Fig. 3. Degrees of circular polarization (P_C) measured as a function of the angle of diamond crystal ($\Delta\theta$).

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References

- [1] M. Suzuki, Y. Inubushi, M. Yabashi and T. Ishikawa: *J. Synchrotron Rad.* **21** (2014) 466.
- [2] K. Hirano *et al.*: *Jpn. J. Appl. Phys.* **30** (1991) L407.

Wavefront measurements by means of X-ray grating interferometry

The unique properties of the X-ray pulses delivered by the SPring-8 Angstrom Compact free electron Laser (SACLA) in terms of photon flux, pulse duration and spatial coherence initiated novel and innovative experiments. Many of these depend on a clean and well-defined wavefront or phase. The conservation of the latter property is, however, one of the most demanding challenges in the design of X-ray beamlines and instrumentation components despite recent progresses. Thus, adequate metrology tools are required to characterize the wavefront under operational conditions in order to support experiments and improvements of the optical components.

In this respect, it was demonstrated that X-ray grating interferometry (XGI) is a valuable technique for spatially resolved *in situ* investigations of the X-ray wavefront at synchrotron beamlines [1] and at hard XFEL facilities [2]. With respect to off-line metrology tools, XGI allows for an at-wavelength characterization of the optical components and to identify in a non-invasive manner eventual radiation related deteriorations of the wavefront properties. We employed the XGI technique, which offers an angular sensitivity of the order of ten nanoradian, to investigate the X-ray wavefront after the optical elements, a pair of total reflection mirrors (TRM) or a Si (111) double crystal monochromator (DCM) [3], installed at beamline **BL3** of SACLA [4].

The principle of XGI is based on the Talbot effect: following the diffraction by a periodic binary grating on which the X-rays are incident, the propagation direction is changed by a small shear angle and at certain, discrete distances downstream of the diffraction grating a constructive interference pattern is created. Any distortion in the wavefront will induce a distortion in the measured interference pattern. The angular sensitivity of the grating interferometer depends on the distance behind the grating at which the interference pattern is measured and inversely on the period of the binary grating. The latter parameter determines also the period of the diffraction pattern which can, given the micro-sized structure of the diffraction grating, not be resolved by position-sensitive detectors. Therefore a second grating, having a period matching the one of the interference pattern, is inserted in front of the detector and used as a transmission mask (Fig. 1), resulting in the creation a pattern of moiré fringes which can be conveniently detected in a single-shot mode.

The employed gratings (Fig. 2) were produced

from thinned Si substrates using electron-beam lithography and deep reactive ion etching. The diffraction grating, having a period of 4 μm , was designed to induce a π -phase shift of the X-ray photons passing through the grating structures and to provide an optimum diffraction efficiency into the $\pm 1^{\text{st}}$ diffraction order. The trenches of the second grating, called absorption grating and having a period of 2 μm , were filled with gold, deposited by electroplating, in order to absorb most of the incident photons and optimize the fringe contrast and visibility. It is important that the used gratings are free of any distortions and defects as these would deteriorate the quality of the measurement and of the extracted results. The grating interferometer was positioned in the experimental hutch 1 at a distance between 12.2 m and 17.2 m downstream of the different optical beamline components and operated in the 11th fractional Talbot order (integrating distance of 220.4 mm for a photon energy of 12.4 keV). The moiré patterns were recorded by a 2D camera system coupled to a YAG screen (effective pixel size of 3.83 μm).

Following a calibration measurement, required for a quantitative analysis, the spatially resolved fringe phase and wavefront propagation angles at the interferometer are extracted from the single-shot moiré fringe patterns through Fourier analysis [5]. Subsequently the equivalent height profile of the investigated optical components in the direction perpendicular to the grating structures is obtained. While the wavefront distortions induced by the optical components are retrieved by taking into account the expected spherical wavefront shape, the individual contributions of the mirrors, respectively the crystals, cannot be separated.

The wavefront aberrations were found to be characterized by a root-mean-square value of

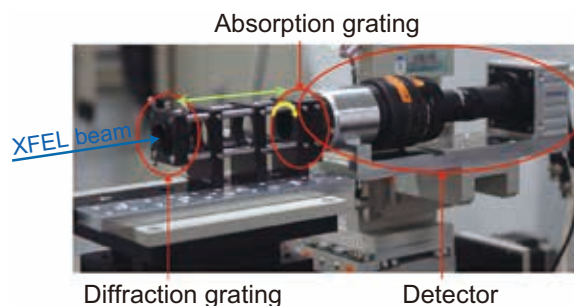


Fig. 1. Picture of the grating interferometry setup.

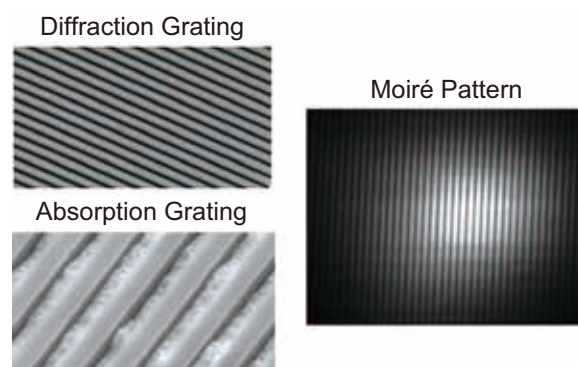


Fig. 2. Scanning electron micrographs of the used grating and example of a recorded moiré pattern.

0.17 μrad or better while the retrieved combined height profiles showed that the installed optical components are of excellent quality [4] (Fig. 3). The differences on the horizontal scale in Fig. 3 are due to different incidence angles of the XFEL beam on the optics. Only at the edges of the mirrors a more pronounced deformation was observed which might be attributed to the mirror mounting. This observation emphasizes the importance of *in situ* measurements

under operational conditions. Moreover, the results confirmed and validated the sensitivity of XGI and its potential as a metrology tool. Indeed, the investigated TRM are among the best currently available mirror (sub-nanometer height variation for an individual mirror). The presented experiment demonstrated thus the potential of XGI to support and contribute to further developments in mirror manufacturing and mirror mounting.

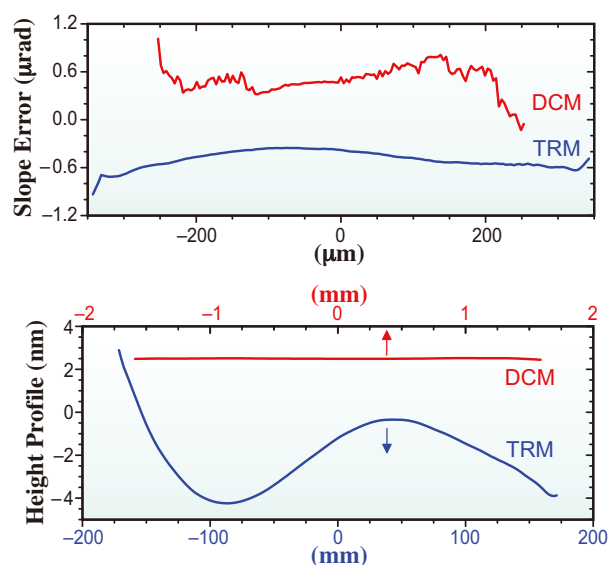


Fig. 3. Aspherical component of the wavefront slope and height profile of the TRM and the DCM extracted from the averaged moiré patterns recorded at an energy of 12.4 keV.

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References

- [1] S. Rutishauser *et al.*: J. Synchrotron Rad. **20** (2013) 300.
- [2] S. Rutishauser *et al.*: Nat. Commun. **3** (2012) 947.
- [3] H. Ohashi *et al.*: Nucl. Instrum. Meth. A **710** (2013) 139.
- [4] Y. Kayser, S. Rutishauser, T. Katayama, T. Kameshima, H. Ohashi, U. Flechsig, M. Yabashi and C. David: Opt. Express **22** (2014) 9004.
- [5] M. Takeda *et al.*: J. Opt. Soc. Am. **72** (1982) 156.

X-ray two-dimensional detector with a multi-port charge-coupled device for X-ray free electron laser experiments

We have developed an X-ray two-dimensional detector equipped with a multi-port readout charge-coupled device (MPCCD) [1,2]. The MPCCD detector was designed to meet experiments performed at the X-ray free electron laser (XFEL) facility SACLA [3]. The detectors are now providing reliable and high-performance X-ray imaging capability. Since user operations began in March 2012, the MPCCD detectors with up to eight-sensor array have been provided.

The design of the MPCCD detector is based on the character of the SACLA XFEL light source. The MPCCD detectors are currently running at a frame rate of 30 Hz, synchronizing to the repetition rate of the present SACLA operation. The detectors also demonstrated to fulfill the demands of recording from a single photon to thousands photons within a several femtosecond pulse and radiation hardness over a nominal annual fluence (1.6×10^{14} photons/mm² at a photon energy of 12 keV).

The CCD sensor is produced with an image format of 1024×512 and pixel size of 50 μm . This design value was determined by referring to the 3D device simulation and the point spread function measurement of the test sensor with a sensing layer thickness identical to the MPCCD sensor. The pixel size was optimized to be small while ensuring that the charge sharing effect is sufficiently suppressed.

Our study of the readout port based on the analytical circuit model yielded the fact that only eight readout ports is enough to operate at maximum frame rate of 60 Hz while achieving peak signal over 4 Me⁻ and the system noise floor of less than 300 e⁻ rms (these correspond to 2400 and 0.18 photons at 6 keV, respectively). This approach has sharply simplified the sensor architecture by reducing so many readout ports to just eight readout ports. The reduction does simplify the associated detector system through simple and robust calibrations, and moderate cooling requirement due to the lower heat dissipation of the sensor. The advantage of this concept was demonstrated by these MPCCD detectors [4].

The deployed detector performance is summarized in Table 1. Figure 1 shows the energy spectra of the Fe55 radiation source measured by the MPCCD detector. The first peak represents the distribution of the electrical noise in the MPCCD detector. The second peak is formed by the X-ray signals emitted from the Fe55 radiation source. The distinct signal from the noise suggests that the MPCCD detector

Table 1. Performance of the deployed MPCCD detector

Description	Deployed performance	Unit
Pixel size	50 × 50	μm
Pixel number	1024 × 512	
Imaging area	51.2 × 25.6	mm ²
Sensing material	Epitaxial Silicon	–
Sensing layer thickness	50	μm
Sensor structure	Front-illumination	–
Image format	Full frame transfer	–
Operation temperature	0 ~ -30	°C
Quantum efficiency	80	% 6 keV
	20	% 12 keV
System noise	100-250	e ⁻
	0.06-0.15	photons@6 keV
Peak signal	4.1-5.0	Me ⁻ /pixel
	2500-3000	photons@6 keV
Radiation hardness	$> 3.2 \times 10^{14}$	photons/mm ² @12 keV
Frame rate	30	Hz
Number of readout port	8	–

is capable of detecting a single photon at a photon energy above 6 keV.

In general, radiation damage affects the threshold voltage in the transistor and the dark current of CCD sensors. To reduce these effects, the MPCCD sensor has a radiation hardened structure in the gate. We evaluated the radiation hardness using beamline BL29XU of SPring-8. The resulting shift in the threshold voltage is sufficiently small to adjust for reliable operation of the MPCCD sensor after irradiation of the nominal annual X-ray dose. The increase in the dark current was also assessed with step irradiation of 3, 9, 30, 60 Mrad (1.6×10^{13} , 4.8×10^{13} , 1.6×10^{14} , 3.2×10^{14} photons/mm² at a photon energy of 12 keV) on to the single MPCCD sensor. The shot noise

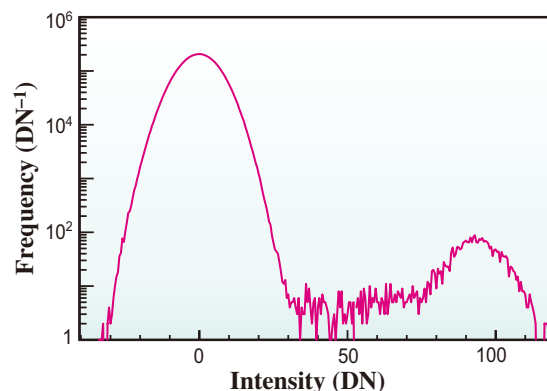


Fig. 1. Energy spectra of the Fe55 radiation source measured by the MPCCD detector.

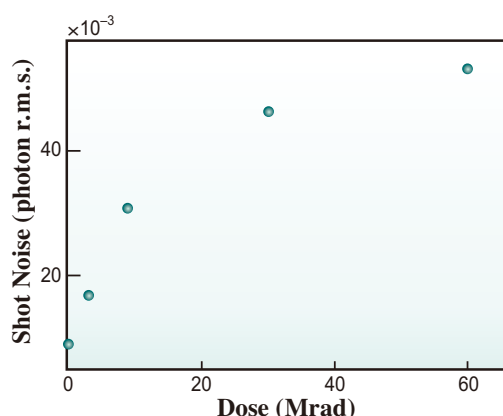


Fig. 2. Dose dependence of the shot noise induced by the dark current of the MPCCD detector. The temperature of the sensor is cooled to be -30°C .

induced by the dark current increases as described in Fig. 2 at a sensor temperature of -30 degree. Even if the accumulated radiation dose to the MPCCD sensor is over two nominal annual dose ($>3.2 \times 10^{14}$ photons/ mm^2 at a photon energy of 12 keV), the effect of the dark current increase on the noise performance is negligible compared with the typical value of the MPCCD system noise of 0.15 photons at a photon energy of 6 keV.

Currently, five kinds of the MPCCD detectors are deployed in user operations at the SACLA as shown

in Fig. 3. They have different camera head structures and image formats, depending on the number of mounting sensors. The MPCCD single and dual with a beryllium window are used in atmospheric conditions. The MPCCD octal is frequently coupled to a user vacuum chamber for coherent diffraction imaging. To deliver high intensity signals close to the diffraction center downstream, a square aperture is equipped in the center of the eight-sensor array. The aperture size is adjustable with up to 8 mm square in a vacuum. Crystallography experiments utilize the MPCCD short working distance (SWD) octal for wide-angle signal measurements. The projecting structure from the base flange permits the sensor array to be close to the sample, allowing the beryllium window to shield the sensor array from contamination arising from sample loading and illumination of optical laser pulses.

At present, the total deployed pixel number is 19.4 Mpixels. The number is scheduled to be increased to 34.1 Mpixels for adequate provision of both the BL3 and the newly constructed BL2, which starts user operations in 2015 (Fig. 3). In summary, the variant camera heads have been stably utilized since user operations began in March 2012 and cover a wide range of the XFEL applications. So far 75% of accepted user proposals at the SACLA utilize the MPCCD detectors as primary data collection apparatus.

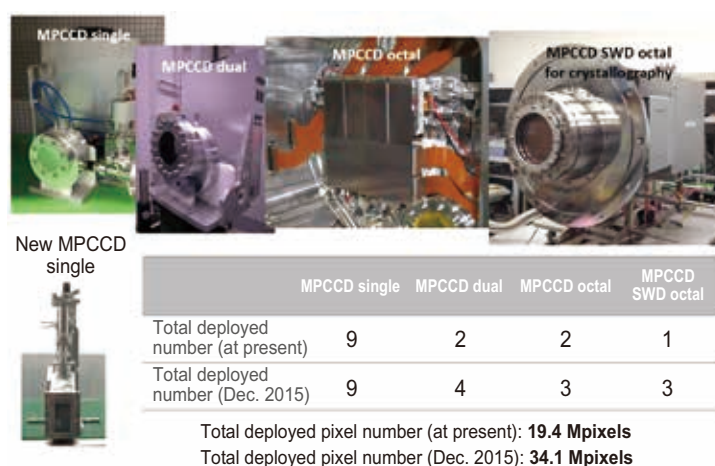


Fig. 3. Summary of the deployable MPCCD detectors.

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References

- [1] T. Kameshima, S. Ono, T. Kudo, K. Ozaki, Y. Kirihera, K. Kobayashi, Y. Inubushi, M. Yabashi, T. Horigome, A. Holland, K. Holland, D. Burt, H. Murao and T. Hatsui: *Rev. Sci. Instrum.* **85** (2014) 033110.
- [2] T. Hatsui: *Synchrotron Rad. News* **27** (2014) 20.
- [3] T. Ishikawa *et al.*: *Nat. Photonics* **6** (2012) 540.
- [4] T. Hatsui and H. Graafsma: *IUCrJ* **2** (2015) 371.

Single shot coherence properties of SACLA in the hard X-ray regime

With the advent of X-ray free electron lasers such as SACLA, chemical and physical properties of matter can be studied that were not accessible before. Many of these applications rely on the coherence properties of the X-ray beam, e.g., imaging of biological macromolecules by coherent diffractive imaging, the study of ultrafast magnetization processes and temporal and spatial correlation spectroscopy. Detailed knowledge of the coherence properties of the X-ray pulses is in particular crucial for experiments on ultrafast dynamics using split-and-delay devices [1]. Due to the stochastic nature of the SASE process, the coherence properties are expected to differ from optical lasers and show shot-to-shot fluctuations. Nevertheless, it is expected that SASE beams show superior transverse coherence. For soft X-rays the degree of coherence is usually studied by double-slit experiments. In the hard X-ray regime this information can be obtained by analyzing diffraction patterns from disordered samples, allowing the determination of coherence properties on a shot-to-shot basis. In our study we measured the coherence properties of SACLA for single shots at a photon energy of 8 keV [2].

The experiment was performed at beamline **BL3** of SACLA in small-angle X-ray scattering geometry. We measured diffraction patterns from amorphous colloidal samples with μm sized X-ray pulses using the MAXIC sample chamber [3]. A sketch from the setup is shown in Fig. 1, including a typical diffraction

pattern recorded with the MPCCD detector. These patterns show a grainy structure in coherent scattering experiments, called speckle pattern. The degree of coherence of the X-ray beam is reflected in the contrast of these speckle patterns [4], given by the normalized standard deviation of the intensity. In this way, we extracted the contrast for a series of 2000 single shots, see Fig. 2(a). The contrast varies between about 0.5 and 1, with an average value of 0.70. We did not observe any significant q -dependence of the contrast (Fig. 2 (b)) within the studied q -window.

The experimentally obtained speckle contrast β_{exp} is defined by the transverse and longitudinal coherence of the X-ray beam and $\beta_{\text{exp}} = \beta_t \beta_l(q)$, with the transverse coherence β_t and a q -dependent correction β_l that depends on the scattering geometry and energy bandwidth. For the experiment at BL3 we found $\beta_t = 0.89$ and thus a transverse coherence of $\beta_t = 0.79 \pm 0.09$ which corresponds to a transversely almost fully coherent beam.

At very low count rates, that are typically present at large q -values and expected for experiments using split-and-delay devices studying sample dynamics down to femtoseconds [1], the presented method for contrast determination breaks down due to statistical limitations. In this case the contrast can be extracted from histograms of the scattered intensity [3]. Experimental intensity histograms are however not discrete because of detector read out noise and charge sharing of neighboring detector pixels.

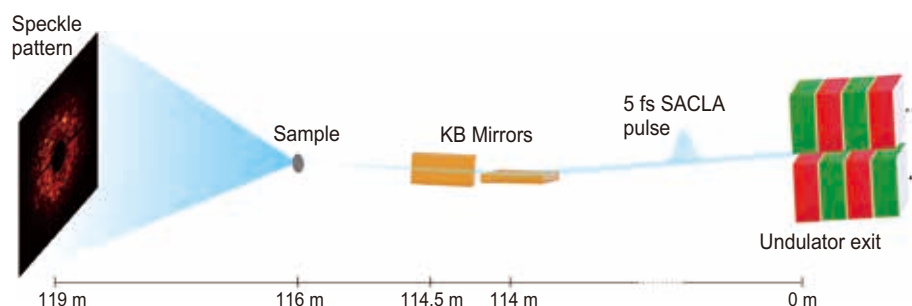


Fig. 1. Schematic set-up of the experiment performed at EH3 of BL3.

Therefore, such data is discretized or a so-called droplet algorithm is used [5], resulting in systematic artifacts or time-consuming analysis procedures, respectively. To overcome these limitations, we developed a simulation model taking detector read-out noise and charge sharing of pixels into account. The intensity histogram of 600 single shots at large q -values is shown in Fig. 3 together with the resulting histogram from simulation. The values obtained within the model resemble the expectations of the detector properties. Most importantly, we obtained the same degree of coherence with both methods.

In conclusion, we determined the degree of coherence at SACLA for hard X-rays on a single shot basis. The transverse coherence was found to be close to 100%, superposed by variations originating from the random SASE fluctuations. Deviations from full transverse coherence depend on set-up and machine parameters such as saturation

lengths, bunch compression schemes and photon wavelength, indicating good agreement of our results with the expectations from FEL theory. Our findings will pave the way for subsequent experiments making use of the coherence properties of SACLA, e.g. studies of ultrafast dynamics via split-and-delay methods.

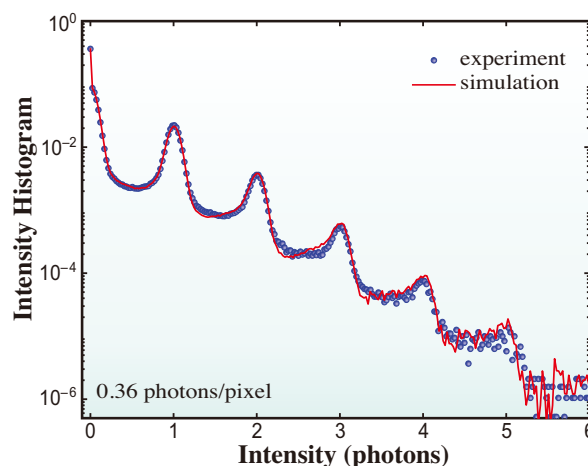


Fig. 3. Intensity histogram at $q=0.07 \text{ nm}^{-1}$ averaged over 600 shots together with simulation results. The intensity is converted to photon number by correcting for the analog-digital-unit of the MPCCD detector.

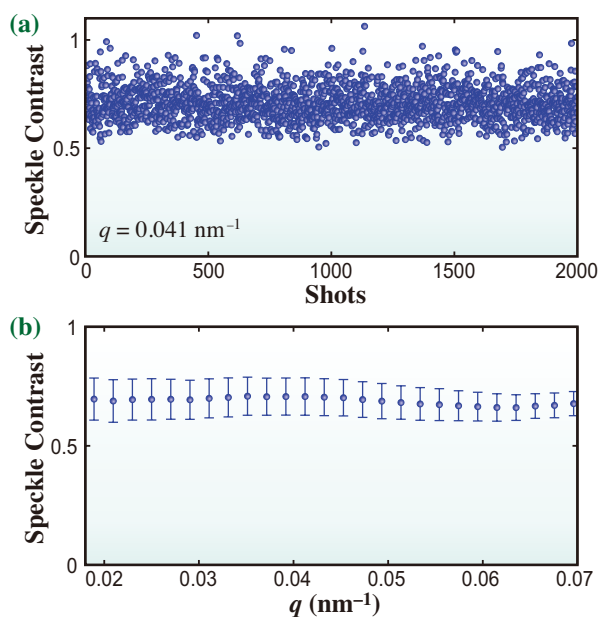


Fig. 2. (a) Single shot speckle contrast from 2000 shots calculated at $q=0.041 \text{ nm}^{-1}$. (b) Contrast as function of q averaged over 2000 shots. The error bars reflect the degree of shot-to-shot fluctuations.

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References

- [1] T. Osaka *et al.*: Proc. SPIE 9210 (2014) 9213009; W. Roseker *et al.*: Proc. SPIE 8502 (2012) 850401.
- [2] F. Lehmkuhler, C. Gutt, B. Fischer, M.A. Schroer, M. Sikorski, S. Song, W. Roseker, J. Glowina, M. Chollet, S. Nelson, K. Tono, T. Katayama, M. Yabashi, T. Ishikawa, A. Robert and G. Grübel: *Sci. Rep.* **4** (2014) 5234.
- [3] C. Song *et al.*: J. Appl. Cryst. **47** (2014) 188.
- [4] C. Gutt *et al.*: Phys. Rev. Lett. **108** (2012) 024801.
- [5] S.O. Hruszkewycz *et al.*: Phys. Rev. Lett. **109** (2012) 185502.

Second harmonic generation at hard X-ray wavelengths

Second harmonic generation (SHG) is a nonlinear effect in which an intense wave at frequency ω interacts with matter to generate another wave at frequency 2ω . It can be viewed as a nonlinear diffraction effect where energy and momentum are conserved.

Nonlinear optical phenomena have been vastly investigated in the last decades at wavelengths ranging from the infrared to the ultraviolet regimes. In this spectral range, concepts and techniques based on nonlinear optics are implemented in many diverse fields. For example, laser technology, optical communication, ultrafast science, quantum optics, and nonlinear spectroscopy techniques.

Due to the weakness of nonlinear interactions in the hard X-ray regime and insufficient peak brightness of all X-ray sources prior to the X-ray free-electron lasers (XFELs), the only nonlinear effect in this regime that has been observed until very recently is spontaneous parametric down conversion [1]. This effect relies on the interaction of the input beam with the large fluctuations of the vacuum field at X-ray wavelengths. The detection scheme, which utilizes fast coincidence measurements, enables the measurement of very low count rates. XFELs provide peak brightness, which is more than 10^{10} times of that of third generation synchrotrons, thus open new possibilities for observations of new nonlinear phenomena. Indeed, the number of publications related to nonlinear X-ray effects is increasing rapidly (for example [2-3]). The nature of X-ray nonlinearities is very different from conventional nonlinearities in many aspects, thus the observations of those effects are interesting and form a basis for new spectroscopy and diffraction techniques.

Nonlinear interactions at photons energies, which are much larger than the electronic resonances, are dominated by free-electron nonlinearities. In the particular case of X-ray SHG, the strong electric field of the pump at frequency ω drives an electron velocity that oscillates at ω . This velocity mixes via the Lorentz force with the magnetic field of the same pump, which also oscillates at ω , to generate nonlinear current density that oscillates at frequency 2ω . The transverse component of the current density gives rise to the second harmonic radiation. The direction of the generated radiation is determined by momentum conservation (phase-matching). At hard X-ray wavelengths, phase matching is achieved by using the periodic structure of the generator crystal.

The phase matching diagram is shown in Fig. 1. The wave vectors of the pump and the second harmonic are $\vec{k}_\omega$, $\vec{k}_{2\omega}$ respectively, and $\vec{G}$ is the reciprocal lattice vector. θ_ω and $\theta_{2\omega}$ denote the angles between the atomic planes and the pump and the second harmonic beams respectively. The phase matching condition for a SHG process at X-ray wavelengths is $2\vec{k}_\omega + \vec{G} = \vec{k}_{2\omega}$.

The experiment was performed at beamline BL3 of SACLA. The incident beam was a 7.3 keV monochromatic beam and the nonlinear medium was a diamond crystal [4]. The average pulse-energy after a Si (111) monochromator was about 1 μ J. The beam was focused by using Kirkpatrick-Baez mirrors to the about 1.5 microns full width at half maximum (FWHM). The phase matching was achieved by slightly detuning the crystal from the orientation for the elastic Laue geometry diffraction from the (220) planes for the second harmonic. The scattered photons were detected by a YAP:Ce scintillation detector (30% energy resolution).

Due to the small-expected efficiency, the main challenge of the experiment was the exclusion of the residual second harmonic that is generated in the XFEL from the SHG measured signal. The most convincing test to distinguish between the contributions of the elastic scattering of the residual second harmonic and of the SHG signal is the dependence of the count rates of the measured signal on the pulse-energy of the input beam. The dependence of the SHG count rates is expected to be quadratic, while elastic scattered signals are linear with the pulse-energy of the input beam. We used two approaches: i) we insert different thicknesses (0.025 mm, 0.1 mm, and 0.2 mm, and no filter) Al foils before

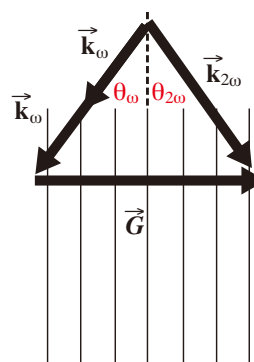


Fig 1. Phase matching diagram.

the diamond crystal and measured the count rates as a function of the average pulse-energy. The results are shown in Fig. 2(a). ii) We used the fluctuations of the XFEL pulse energy by building histograms of the number of pulses at a given pump pulse-energy. We scanned through the recorded data and counted the number of photons measured within each pump pulse-energy bin. We calculated the count rate by dividing the number of the second harmonic counts in each bin by the number of pulses with pulse-energy within the bin. The results are shown in Fig. 2(b). The inset shows the histogram of the pump-pulse energies. The results of the two approaches show clearly the quadratic dependence of the second harmonic signal on the pump, and agree within the experimental uncertainties.

Figure 3 shows the rocking curve of the SHG process, namely, the second harmonic count rate as a function of the angular deviation of the crystal from the phase matching angle. The Gaussian fit reveals that the FWHM of the rocking curve is about 180 μ rad. The rocking curve confirms that the SHG is observed only in a narrow angular range near the phase matching angle.

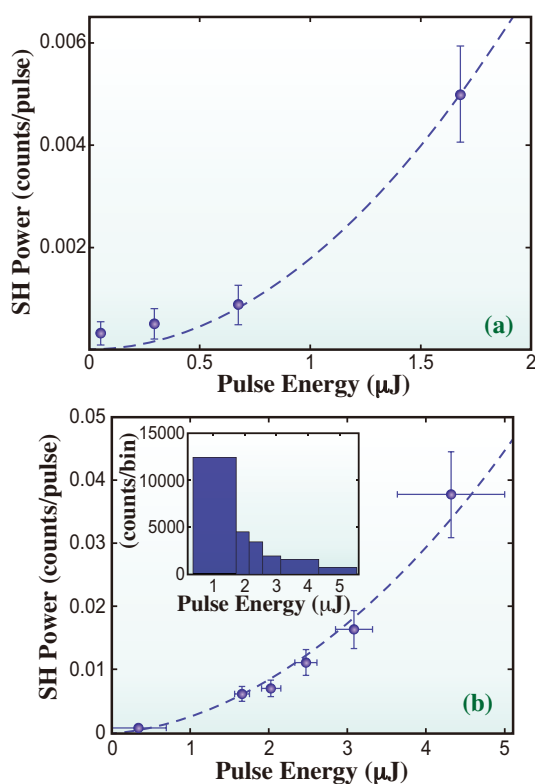


Fig. 2. Second harmonic count rate as a function of (a) Average pump pulse-energy where the pulse-energy is varied by inserting thin Al filters before the diamond crystal (b) The pulse-energy dependence is obtained from the pulse-energy fluctuations of the XFEL beam. The inset shows the energy fluctuations of the XFEL beam.

In conclusion, we have observed the first SHG at hard X-ray wavelengths. As expected, the power of generated second harmonic scales quadratically with the pulse-energy of the pump, and the highest efficiency is obtained at the phase matching angle. The largest observed SHG efficiency was $5.8 \pm 1.1 \times 10^{-11}$ leading to a count rate that is more than ten times above the background. The observation of X-ray SHG opens possibilities for more general nonlinear studies at X-ray wavelengths. For example, third harmonic generation, difference frequency generation, and the generation of quantum states of light via parametric down-conversion. SHG at X-ray wavelengths can be used in temporal correlation measurements for diagnostics of XFELs pulses on the femtosecond to sub-femtosecond scales and for nonlinear microscopy.

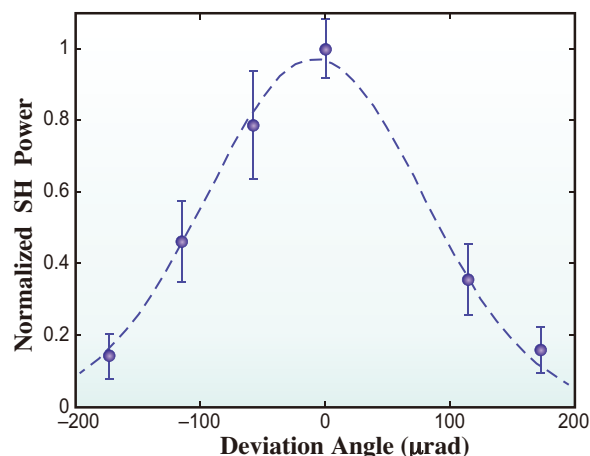


Fig. 3. Rocking curve of the second harmonic signal.

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References

- [1] B.W. Adams *et al.*: J. Mod. Opt. **60** (2012) 2.
- [2] T.E. Glover *et al.*: Nature **488** (2012) 603.
- [3] K. Tamasaku *et al.*: Nat. Photonics **8** (2014) 313.
- [4] S. Schwartz, M. Fuchs, J.B. Hastings, Y. Inubushi, T. Ishikawa, T. Katayama, D.A. Reis, T. Sato, K. Tono, M. Yabashi, S. Yudovich and S.E. Harris: Phys. Rev. Lett. **112** (2014) 163901.

Femtosecond time-resolved X-ray absorption spectroscopy using SACLA: Dual beam direct absorption and X-ray fluorescence

X-ray absorption spectroscopy (XAS) interrogates inner-shell electrons strongly bound to nuclei, enabling element-specific investigations of the electronic and geometrical structures of molecules. The ionization energy of an inner-shell electron, which is observed as the absorption edge, varies with the atomic number and exhibits a “chemical shift,” revealing the oxidation state and/or chemical bonding of the atom. An X-ray absorption spectrum also displays oscillatory features, termed the extended X-ray absorption fine structure (EXAFS), in the post-edge region owing to scattering of a photoelectron by neighboring atoms. EXAFS analysis provides the bond lengths around the atom of interest. SACLA generates an X-ray pulse as short as 10 fs with a pulse energy on the order of μJ , opening new avenues for femtosecond X-ray spectroscopy. Considering the low repetition rates of SACLA (30 – 60 Hz), time-resolved XAS (TRXAS) is one of the best-suited techniques.

We started our project supported by the “X-ray Free Electron Laser Priority Strategy Program” of the Ministry of Education, Culture, Sports, Science, and Technology of Japan (MEXT) in 2012, and have succeeded in TRXAS using a dual beam multiplex detection method as shown in Fig. 1 in 2013 [1,2]. In this method, a transmission grating splits a hard X-ray pulse (FWHM = 30 eV) into two equally intense beams. One of which is transmitted through a sample solution and used as a signal beam. The signal beam and the reference beam, which does not pass through the sample, are spectrally dispersed and simultaneously recorded on a shot-to-shot basis. From these signal and reference spectra, an X-ray direct absorption spectrum is obtained for the entire

30 eV spectral bandwidth of the X-ray pulse.

While the simultaneous measurement of an absorption spectrum for a 30-eV wide energy region provides a high efficiency, the direct absorption method inherently suffers from low sensitivity. Therefore, we have implemented TRXAS using X-ray fluorescence detection method, which provides an order of magnitude higher detection sensitivity than the dual beam method (Fig. 2) [3]. X-ray radiation from SACLA is monochromatized to 1.3 eV using a Si(111) X-ray monochromator, and focused on the sample using Kirkpatrick-Baez mirrors down to a spot diameter of 1.5 μm . A sample is photoexcited with a femtosecond UV pulse (268 nm) generated by a Ti:sapphire regenerative amplifier, and a transient X-ray absorption spectrum of the excited species is measured by scanning the photon energy of the time-delayed X-ray pulse while monitoring X-ray fluorescence with a multi-port charge-coupled device sensor.

Figure 3(a) shows the transient X-ray absorption spectra, which are observed using X-ray fluorescence, of aqueous 0.1 M ammonium iron(III) oxalate trihydrate solution at selected pump-probe delay times. The spectra reveal a clear change that occurs instantaneously upon UV irradiation, while the subsequent spectral evolution is rather small. The difference spectra shown in Fig. 3(b) were calculated from the spectra shown in Fig. 3(a) by subtracting the spectrum measured at -3 ps from those measured at positive time delays. The difference spectra reveal that the largest spectral change upon UV irradiation is the red-shift of the *K*-edge. Magnification of the *K*-edge region, shown in Fig. 3(c), reveals that the

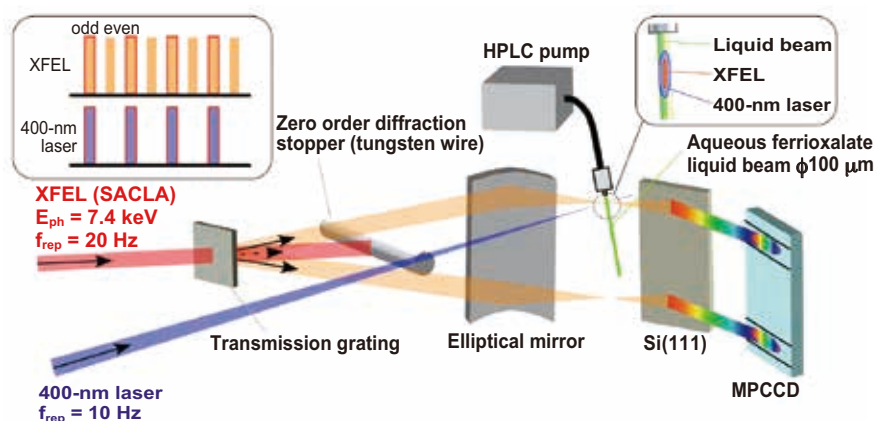


Fig. 1. Dual beam multiplex detection method.

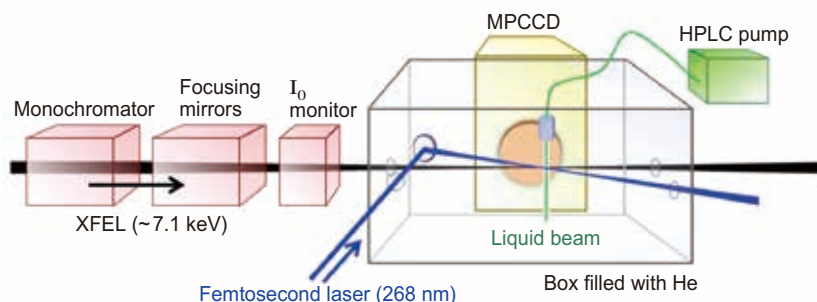


Fig. 2. X-ray fluorescence method.

red-shift of the *K*-edge diminishes by more than 2 eV within 3 ps.

From the transient spectra, we extracted the spectrum of photochemical reaction product. The red-shift of Fe *K*-edge indicates that Fe(III) is reduced to Fe(II) by the ligand-to-metal charge transfer, and

the reduction of the oscillatory feature in the product spectrum suggests that one ligand dissociates within 5 ps after the pump pulse. This method can be used to study the carrier dynamics and redox reactions of various samples. We are also planning to improve the sensitivity further for biological samples.

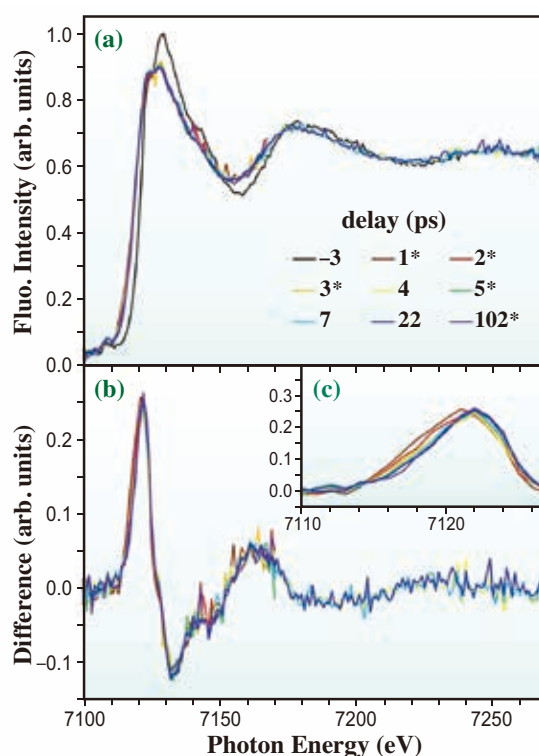


Fig. 3. (a) Time-resolved X-ray absorption spectra of an aqueous $[\text{Fe(III)(C}_2\text{O}_4)_3]^{3-}$ solution observed at various time delays. Excitation wavelength is 268 nm. (b) Difference spectra between positive delay times and -3 ps. (c) Enlarged view of the reddest part of the difference spectra, which reveals that the magnitude of the red-shift diminishes within 3 ps.

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References

- [1] T. Katayama *et al.*: Appl. Phys. Lett. **103** (2013)131105.
- [2] Y. Obara *et al.*: Opt. Express **22** (2014) 1105.
- [3] Y. Ogi, Y. Obara, T. Katayama, Y.-I. Suzuki, S.Y. Liu, N.C.-M. Bartlett, N. Kurahashi, S. Karashima, T. Togashi, Y. Inubushi, K. Ogawa, S. Owada, M. Rubešová, M. Yabashi, K. Misawa, P. Šlavíček and T. Suzuki: Struct. Dyn. **2** (2015) 034901.

Femtosecond single-shot imaging with MAXIC at SACLA

X-ray Free-Electron Lasers (XFELs) are a new generation of X-ray sources that produce ultrafast ($\sim 10^{-14}$ s) and ultra bright ($\sim 10^{12}$ photons per pulse) coherent X-ray pulses that allow for unique and innovative experimentation. However, in contrast to synchrotrons, where multiple end stations are available, XFELs are single user facilities. Given the current limited availability of XFEL sources, experimental platforms at these facilities should be adaptable, accommodating both novel and routinely requested experimental set-ups. To this end we have developed the Multiple Application X-ray Imaging Chamber (MAXIC), a generic platform for performing a wide variety of single-shot, coherent diffraction experiments at SACLA [1]. Here the current status of the chamber will be outlined and recent successful experiments using the MAXIC will be highlighted [2].

The MAXIC is currently available for installation in EH3 of BL3 at SACLA, as shown in Fig. 1. The main chamber is compact, with several flanges and ports compatible with beamline components such as; a high power optical laser, Multiport CCD (MPCCD) detector and focusing mirrors [1]. Inside the chamber are several high precision XYZ motor stages (Kohzu Precision Co.) for alignment of samples, optical and pulse characterization instrumentation such as a set of 4-way cross slits, a Ce:YAG screen and a 50 μm gold cross wire.

XFELs provide a means to overcome traditional radiation damage barriers in diffraction experiments. Their high peak brilliance and short pulse duration allow snapshot diffraction patterns to be captured from samples faster than radiation induced damage can propagate [3]. This requires a high sample renewal rate, delivering a fresh sample to each pulse. The MAXIC can facilitate several single-particle delivery schemes outlined in Figs. 1(b-d), categorized as either “fixed” or “flying” [1]. “Fixed” targets are generally randomly dispersed onto thin, multi-windowed Si_3N_4 membranes. Raster scanning across the XFEL focal point, whilst synchronizing with a fast shutter, ensures a single exposure at each scan position. “Flying” samples are delivered to the XFEL focal spot in a continuous particle stream. They may be delivered in a gas focused liquid jet, ideal for serial crystallography, or in the gas-phase via an aerodynamic lens stack (ADL), preferable where low background noise is essential.

The true test of an experimental platform, however, is in its application to novel scientific problems. We

have employed the MAXIC in experiments probing the nano-scale structure of a self-assembling, micron-sized macromolecule, the RNA interference (RNAi) microsphere [4]. The microspheres are composed entirely of long RNA strands and each strand contains multiple copies of a short interfering RNA (siRNA) sequence, which can be cleaved from the sphere to enter the RNAi pathway and alter expression of target genes [4]. Previously published work focused predominantly on the surface morphology of the microspheres, however for a more detailed understanding of its structure and function, characterization of internal morphology is also necessary. We applied the Coherent Diffractive Imaging (CDI) technique in this study as it allows quantitative views of electron density variation in micron thick samples without the need for sectioning or labeling [5].

CDI experiments were performed at beamlines BL3 of SACLA and BL29XU of Spring-8, the results of which are shown in Fig. 2. RNAi microspheres

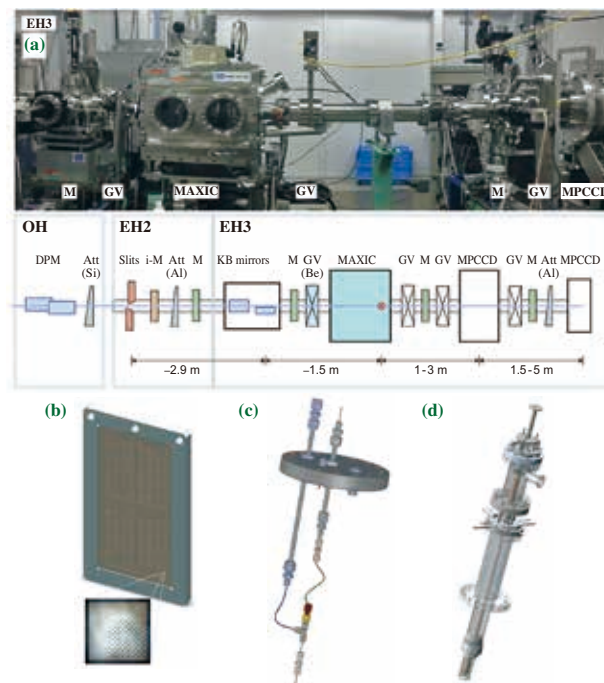


Fig. 1. Installation of the MAXIC at BL3 and key components. (a) Photograph of the MAXIC installed at EH3 of BL3 (top) and the overview of total layout within BL3 (bottom). M and GV represent beam monitor and gate valve positions respectively. Red circle represents the XFEL focal spot. (b) Fixed target sample holder with two multi-window Si_3N_4 membranes attached and a photograph of a single membrane after exposure to XFEL pulses (inset). (c) Flexible liquid jet injector. (d) Gas-phase injector with ADL stack.

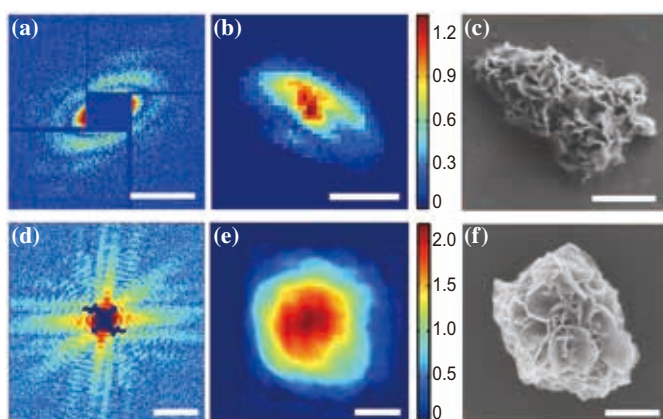


Fig. 2. Coherent diffraction imaging experiments at SACLA and SPring-8. (a) Single-shot diffraction pattern of an isolate microsp sponge. (b) Reconstructed image. (c) SEM Electron micrograph of a similar microsp sponge. (d) Averaged diffraction pattern of an isolate microsp sponge. (e) Reconstructed image. (f) SEM Electron micrograph of a similar microsp sponge. Color map represents relative electron density. Scale bars represent $10 \mu\text{m}^{-1}$ (a and d) 250 nm (b) and 500 nm (c, e and f).

were prepared according to published methods [4] and dispersed upon multi-window Si_3N_4 membranes. These were then mounted either in the MAXIC or a synchrotron based CDI chamber. For single-shot experiments samples were exposed to $2 \mu\text{m}$ focused, 5.5 keV pulses from SACLA. A far-field diffraction pattern from an individual microsp sponge was captured (Fig. 2(a)) and its electron density reconstructed via iterative phase retrieval algorithms (Fig. 2(b)). The image shows a progressive change in internal density from a high density central region. This conforms

well with images reconstructed from full-sized microsponges analyzed at SPring-8 (Figs. 2(d-e)).

To gain greater insight into these internal features Coherent Diffraction Tomography (CDT) was performed at BL29XU. Here diffraction patterns are collected over a $\pm 69^\circ$ tilt series and combined to reconstruct a complete 3D electron density. The results in figure 3 display a distinct high density “core-like” region buried within the microsp sponge. The average electron density of this region is 0.54 e^- per $\text{\AA}^3$, close to the nominal electron density of RNA of 0.55 e^- per $\text{\AA}^3$, suggesting that it is composed almost exclusively of densely packed RNA strands [2]. To further confirm this feature coherent SAXS (cSAXS) experiments were performed at SACLA. A sample cSAXS pattern, captured from a cluster of RNAi microsponges, is shown in Fig. 3(b). The pattern was radially averaged to produce a 1D curve and then this was fit to a simple core-shell ellipsoid model, based upon observations from CDI, as shown in Fig. 3(c). The model shows a consistent fit to the experimental data, with a normalized r.m.s. error of 0.8% , suggesting that the core-shell morphology is a generic feature of the RNAi microsp sponge [2].

In summary we have developed a novel platform for a variety of coherent X-ray diffraction experiments at SACLA. We have demonstrated an analysis scheme for combining single-shot experiments at an XFEL with more conventional CDI experiments at a synchrotron. We have applied this scheme to uncover the core-shell morphology of a novel biomolecular construct, the RNAi microsp sponge.

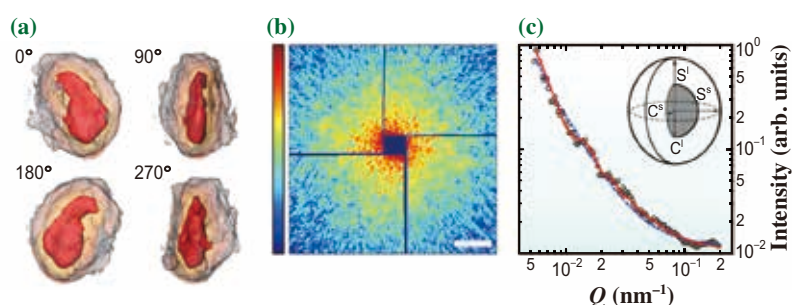


Fig. 3. Coherent diffractive tomography and cSAXS. (a) Reconstructed 3D electron density at various rotation angles relative to the y axis. (b) Sample cSAXS pattern. Color map represents relative intensity. (c) 1D scattering curve fit to a simple ellipsoid model (blue dashed line) and core-shell ellipsoid model (red line, model inset).

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References

- [1] C. Song, K. Tono, J. Park, T. Ebisu, S. Kim, H. Shimada, S. Kim, M. Gallagher-Jones, D. Nam, T. Sato, T. Togashi, K. Ogawa, Y. Joti, T. Kameshima, S. Ono, T. Hatsui, S. Iwata, M. Yabashi and T. Ishikawa : *J. Appl. Cryst.* **47** (2014) 188.
- [2] M. Gallagher-Jones *et al.*: *Nat. Commun.* **5** (2014) 3798.
- [3] R. Neutze *et al.*: *Nature* **406** (2000) 752.
- [4] J.B. Lee *et al.*: *Nat. Mater.* **11** (2012) 316.
- [5] J. Miao *et al.*: *IEEE J. Sel. Top. Quant. Electron.* **18** (2012) 399.

Imaging live cells by X-ray laser diffraction

Radiation damage is a serious problem in high-resolution biological imaging. It has been impossible to observe living cells at a nanometer resolution by electron microscopy or X-ray microscopy because cells die when exposed to the electrons or X-ray radiation used for the observations. X-ray free-electron lasers (XFELs) can overcome this difficulty by illuminating the sample with ultra-short femtosecond pulses, allowing a snapshot of the intact sample to be acquired before radiation damage [1,2].

To capture ultrafast snapshots of a solution sample at the nanometer resolution using XFELs, we developed a method, which we refer to as pulsed coherent X-ray solution scattering (PCXSS) [3,4]. Figure 1 shows the PCXSS measurement schematically. Water is essential to life. Hence, solution imaging by PCXSS is crucial in biological imaging. In PCXSS, the sample image is reconstructed from the coherent X-ray diffraction (CXD) pattern using lens-less coherent diffractive imaging techniques [5].

PCXSS has a unique feature; the solution sample is placed statically between two thin membranes in a controlled environment. Since XFEL pulses are intense enough to break the membranes in a single shot, we designed a micro-liquid enclosure array (MLEA) chip where many independent micro-liquid enclosures are arranged in a 2D array as shown in Fig. 2(a). The MLEA chips are fabricated by a photolithography process at Hokkaido University using equipment in a clean room operated with support from the Nanotechnology Platform Project of MEXT, Japan.

As the first demonstration of PCXSS measurement, we performed nano-imaging of a living *Microbacterium lacticum* cell. *M. lacticum* is a heat-stable bacterium discovered in milk. It has a typical rod shape, but

is much smaller than ordinary bacteria. Because conventional optical microscopy has an insufficient resolution to observe the internal structures of a sub-micrometer sized cell, little information is available about its cell biology despite its importance in agriculture.

Before performing an XFEL experiment at SACLA, we confirmed that the cells are alive in the MLEA chip. We stained the cells with fluorescent dyes, and observed them by fluorescent microscopy. In Fig. 2(b), live cells are labelled in green and the dead ones are in red. Our experiment shows that 99% of the cells are alive in an MLEA chip one hour after enclosure being placed in the vacuum environment, indicating that MLEA chips are adequate for live cell imaging by PCXSS.

The PCXSS measurement of live *M. lacticum* cells was carried out at beamline BL3 of SACLA. The XFEL beam from the undulator with a photon energy of 5.5 keV was first transported to the optics hutch, and higher harmonics were removed with a pair of flat total reflection mirrors there. The beam was then guided to EH3 and focused onto a $1.5\ \mu\text{m} \times 2.0\ \mu\text{m}$ spot using a Kirkpatrick-Baez mirror system. At the focus, the XFEL beam illuminates *M. lacticum* cells in a 0.9% (w/v) NaCl solution in an MLEA chip placed in MAXIC (Multiple Application X-ray Imaging Chamber). The CXD patterns of the sample were recorded by MPCCD (Multi-Port Charge-Coupled Device).

Because X-ray scattering from a sub-micrometer sized biological sample is extremely weak, it is important to reduce parasitic background scattering, originating from something other than the sample, for high precision measurements. Consequently, we placed two sets of four-jaw guard slits before the

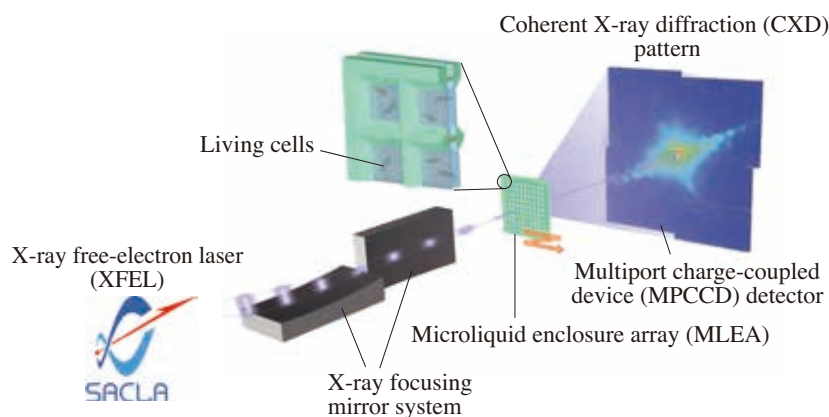


Fig. 1. Schematic of the PCXSS experiment.

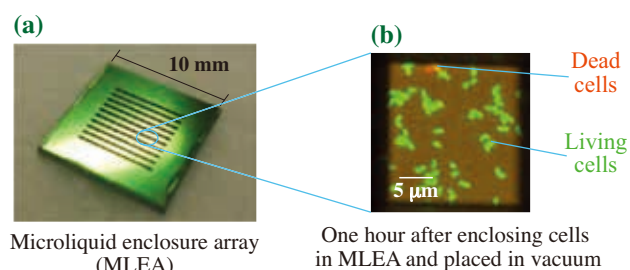


Fig. 2. (a) MLEA chip. **(b)** Result of fluorescence microscopy study confirming that cells can be kept alive in MLEA chips.

sample in MAXIC. Moreover, we confirmed that the silicon window frame of an MLEA chip with an aperture size of $\sim 20 \mu\text{m}$ acts as an additional guard slit in the sample plane, and plays an essential role in reducing parasitic scattering.

Figure 3(a) shows a single-shot CXD pattern of a live *M. lacticum* cell. An interference fringe extending in one direction is clearly observed. The fringe interval indicates that the sample has a width of 194 nm. Figure 3(b) shows the reconstructed sample image. In the image reconstruction, we used the relaxed averaged alternating reflections (RAAR), the noise-

tolerant hybrid input-output (HIO), and shrink-wrap algorithms. The full-period resolution of the image, which was estimated using the phase-retrieval transfer function, is 37 nm.

The reconstructed cell image is rod-shaped with a width and length of $\sim 194 \text{ nm}$ and $\sim 570 \text{ nm}$, respectively. The lower part of the cell image contains a dumbbell-shaped high image-intensity region, indicative of a nucleoid, a DNA-rich structure in prokaryotic cells. In fact, the image intensity difference between the upper and lower regions of the cell can be roughly explained by assuming that they are mostly composed of protein and nucleic acids, respectively.

Our research demonstrates that the PCXSS method using XFEL has great potential for observing biological samples close to their natural state at a nanoscale resolution without staining with heavy metals or thin sectioning. As the achieved resolution greatly exceeds conventional optical microscopy, PCXSS should help reveal important cellular events, such as genome replication and subsequent cell division. By further improving the resolution, nanostructures of natural-state biological molecules will be also imaged. In addition, PCXSS can be usefully applied to imaging nanostructures of materials functioning in solution.

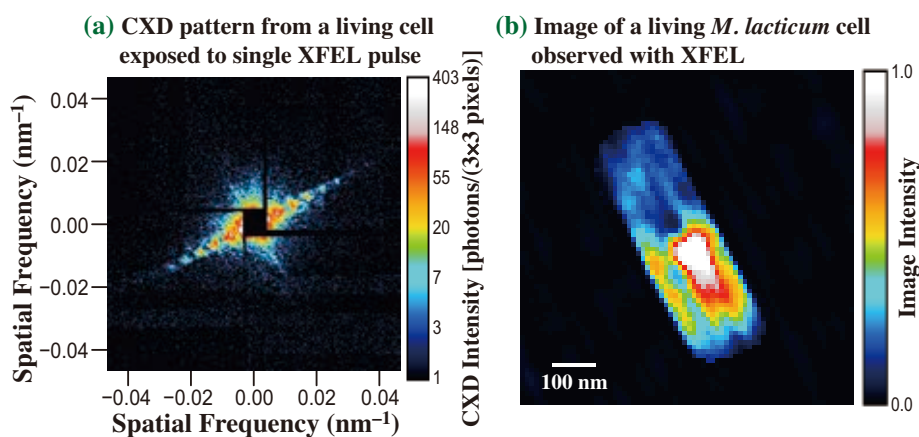


Fig. 3. (a) Single-shot CXD pattern of a living *M. lacticum* cell. **(b)** Reconstructed image obtained by applying iterative phase retrieval algorithms to the CXD pattern.

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References

- [1] R. Neutze *et al.*: Nature **406** (2000) 752.
- [2] H.N. Chapman *et al.*: Nat. Phys. **2** (2006) 839.
- [3] J. Pérez and Y. Nishino: Curr. Opin. Struct. Biol. **22** (2012) 670.
- [4] T. Kimura, Y. Joti, A. Shibuya, C. Song, S. Kim, K. Tono, M. Yabashi, M. Tamakoshi, T. Moriya, T. Oshima, T. Ishikawa, Y. Bessho and Y. Nishino: Nat. Commun. **5** (2014) 3052.
- [5] J. Miao *et al.*: Nature **400** (1999) 342.

KOTOBUKI-1 apparatus for cryogenic coherent X-ray diffraction imaging

Coherent X-ray diffraction imaging (CXDI) is a technique to visualize three-dimensional structures of non-crystalline particles with micrometer to sub-micrometer dimensions. In CXDI experiment, an isolated particle is irradiated by X-rays with a high transverse coherence, and then the Fraunhofer diffraction pattern is recorded so that the number of pixels in the detector is more than the twice that in the electron density map of the particle at a desired resolution (oversampling). The electron density map is, in principle, reconstructed by applying the phase-retrieval algorithm to the oversampled diffraction pattern. The weak electromagnetic interaction of hard X-rays with atoms allows the long penetration depth to visualize thick specimens, which are too opaque for electron microscopy without multiple scattering.

One application of CXDI is the structure analyses of cellular components, such as organelles and whole cells, which have never been crystallized. Biological specimens should be kept in a fully hydrated state to visualize their functional structures. Cryo-cooling of specimens used in electron microscopy and protein crystallography avoid degradation in a vacuum environment, which is necessary to reduce background scattering, and radiation damage in synchrotron experiments. Diffraction data collection at liquid nitrogen temperatures dramatically reduces radiation damage of specimens. In addition, flash-cooling allows the functional structures of specimens to be observed even under a vacuum [1,2].

We developed a diffraction apparatus named KOTOBUKI-1 (Fig. 1) for cryogenic CXDI experiments of frozen-hydrated non-crystalline particles at SPring-8 and SACLA [1]. The apparatus is composed of three major components: a vacuum chamber equipped with a cryogenic pot connected to a goniometer, a loading device to transfer frozen-hydrated specimens to the pot, and an alignment table to mount the chamber and the loading device. The cryogenic pot can be operated at 66 K through the evaporation cooling effect of liquid nitrogen, suppressing the positional fluctuations of specimens to less than 0.4 μm . A set of loading devices enables the specimen holders stored in liquid nitrogen to be mounted onto the specimen stage in the vacuum chamber.

Recently, we have been using the apparatus together with two multi-port CCD detectors for single-shot XFEL-CXDI experiments of non-crystalline particles with the dimensions of 100 – 1000 nm at beamline BL3 of SACLA. We usually use X-rays with

the photon energy of 5.5-keV and the intensity of $10^{10} - 10^{11}$ photons/ μm^2 /pulse. Because an extremely intense X-ray pulse destroys particles at the atomic level, the specimen disk is raster-scanned by the goniometer to supply fresh particles to the irradiation area. Collected diffraction patterns are processed using the program suite SITENNO [3,4]. Immediately after completing each data collection run, SITENNO automatically selects diffraction patterns with a significant level of intensity and conducts phase retrieval calculations [5-8].

As illustrated in Fig. 2, the diffraction pattern from cube-shaped particles with dimensions of 500 nm is approximated with the Fraunhofer diffraction pattern expected from a square-shaped aperture. This finding indicates that diffraction from the particle occurs before

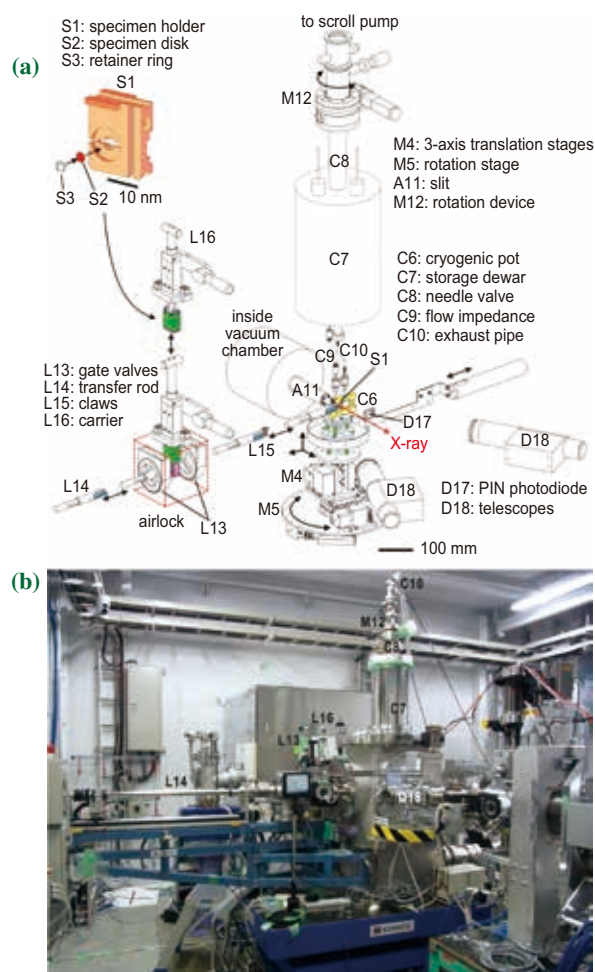


Fig. 1. (a) Illustration showing the arrangement of key components and (b) photograph of the KOTOBUKI-1 apparatus viewed downstream.

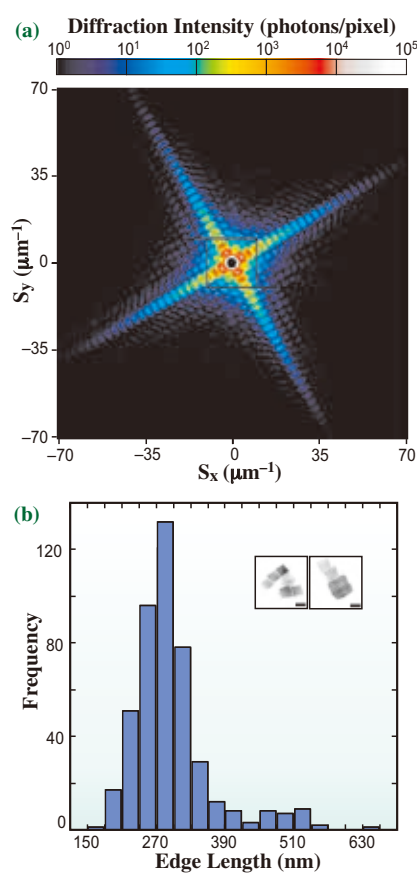


Fig. 2. (a) Diffraction pattern from a cube-shaped copper-oxide particle and (b) the size distribution determined from the electron density maps of 1276 particles.

the destruction. The XFEL-CXDI experiment allows us to collect a huge number of diffraction patterns within a short time. As an application, we tried to determine the size distribution of synthesized nano-metal particles. Even from aggregates of several particles, the phase-retrieval software robustly reconstructs the projection electron density maps as displayed in the upper half of Fig. 3 [3,7]. The distribution is consistent with that from scanning electron microscopy prior to the XFEL-CXDI experiment [9,10].

In addition to applications for material sciences and industry, XFEL-CXDI allows the structure of organelles in biological cells to be visualized. Organelles are dispersed on thin membranes with a small amount of buffer solutions to reduce background scattering and to ensure the contrast in the projected electron density maps. When the number density is $10/10 \times 10 \mu\text{m}^2$, the hit rate by XFEL pulses exceeds 50%. Recently we successfully visualized the electron density distribution in chloroplasts and nuclei of eukaryote cells [11]. XFEL-CXDI would become a suitable tool to visualize intact and whole organelles in addition to sophisticated imaging techniques such as

fluorescence microscopy, electron microscopy, X-ray microscopy, etc. The complementary use of XFEL-CXDI together with these techniques is expected to bridge the resolution gap between cell biology and structural biology.

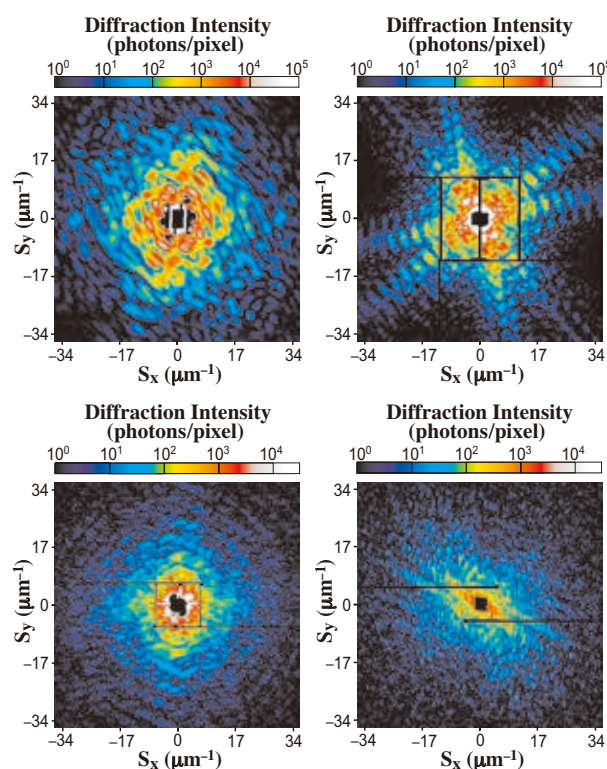


Fig. 3. Representative diffraction patterns observed in XFEL-CXDI experiments for aggregates of synthesized non-metal particles (upper panel) and for organelles of eukaryote cells.

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References

- [1] M. Nakasako *et al.*: Rev. Sci. Instrum. **84** (2013) 093705.
- [2] Y. Takayama & M. Nakasako: Rev. Sci. Instrum. **83** (2012) 054301.
- [3] Y. Sekiguchi *et al.*: J. Synchrotron Rad. **21** (2014) 600.
- [4] Y. Sekiguchi *et al.*: J. Synchrotron Rad. **21** (2014) 1378.
- [5] W. Kodama & M. Nakasako: Phys. Rev. E **84** (2011) 021902.
- [6] T. Oroguchi & M. Nakasako: Phys. Rev. E **87** (2013) 022712.
- [7] A. Kobayashi *et al.*: Opt. Exp. **22** (2014) 27892.
- [8] Y. Takayama *et al.*: Sci. Rep. **5** (2015) 8074.
- [9] Y. Takahashi *et al.*: Nano Lett. **13** (2013) 6028.
- [10] R. Xu *et al.*: Nat. Commun. **5** (2014) 4061.
- [11] Y. Takayama *et al.*: Plant Cell Physiol. (2015) - in press.

Single-shot 3D structure determination of nanocrystals using femtosecond X-ray free electron laser pulses

Since the first experimental demonstration in 1999 [1], the field of coherent diffractive imaging (CDI) has grown rapidly. CDI has been applied to study a wide range of samples using synchrotron radiation and X-ray free electron lasers (XFELs). Recently, we reported the first experimental demonstration of single-shot 3D structure determination of gold nanocrystals at ~ 5.5 nm resolution using ~ 10 femtosecond SACLA pulses [2]. To achieve this, we recorded coherent diffraction patterns from high-index-faceted gold nanocrystals struck by a single SACLA pulse at beamline **BL3**. The high energy of these pulses destroyed the crystals, but in the brief time before their destruction, enough diffracted signal was collected from the crystals to determine their three-dimensional structure. Obtaining a three-dimensional structure from a single view required knowledge and application of the special symmetry present in the nanocrystals. Combined with the unique effect of curvature also intrinsic to the recorded diffraction patterns [3], we reconstructed the 3D structure of each nanocrystal from a single diffraction pattern. Our 3D reconstruction showed, in unprecedented detail, the high-index facets of these nanocrystals.

To further improve the reliability of our results, we combined single-shot diffraction patterns of identical gold nanocrystals. From these, we assembled a 3D diffraction pattern, which produced an average

3D reconstruction. The resolution achieved by this reconstruction is presently limited by the detector geometry. These results present a significant advance in CDI. At a spatial resolution of ~ 5.5 nm in 3D, these reconstructions enjoy the highest resolution ever achieved by any non-crystallographic 3D X-ray imaging method. As symmetry exists in many nanocrystals and virus particles, this method can be broadly applied to 3D structure studies of other such particles at nanometer resolution on femtosecond time scales. Given a sufficient number of identical nanocrystals, this approach can in principle be used to determine the 3D structure of nanocrystals at atomic resolution.

Figure 1 shows the schematic layout of our experimental setup. SACLA pulses were focused onto a small ~ 1.5 μm area, producing a total of 126,976 diffraction patterns from which 1,877 high quality patterns were selected. Figure 1 shows a representative high quality single-shot diffraction pattern after data analysis.

Trisoctahedral nanocrystals belong to the crystallographic point group $m\bar{3}m$, consisting two-, three-, and four-fold rotational symmetries along the $\langle 011 \rangle$, $\langle 111 \rangle$ and $\langle 001 \rangle$ directions respectively, for which there exist a total of 48 symmetry operations used to produce 3D patterns. Using these symmetrized patterns, 3D phase retrieval of

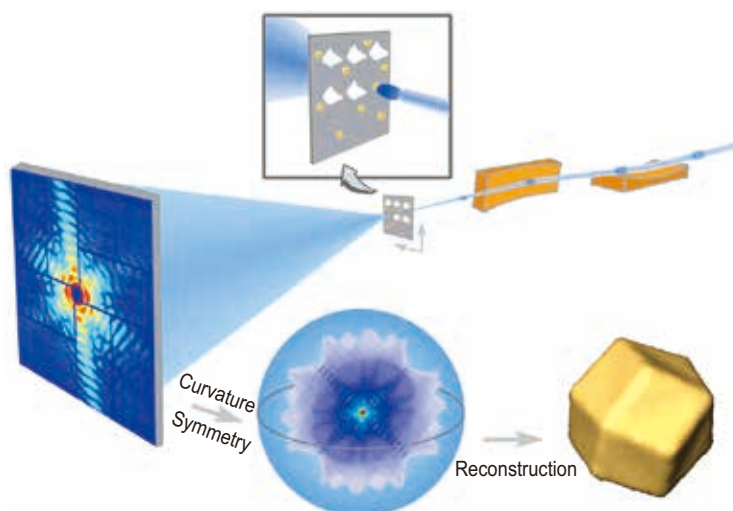


Fig. 1. Schematic layout of the single-shot 3D structure determination using femtosecond XFEL pulses. By using the symmetry of the nanocrystal and the curvature of the Ewald sphere, a 3D diffraction pattern was generated from a single-shot diffraction pattern, from which a 3D reconstruction was computed with a resolution of ~ 5.5 nm.

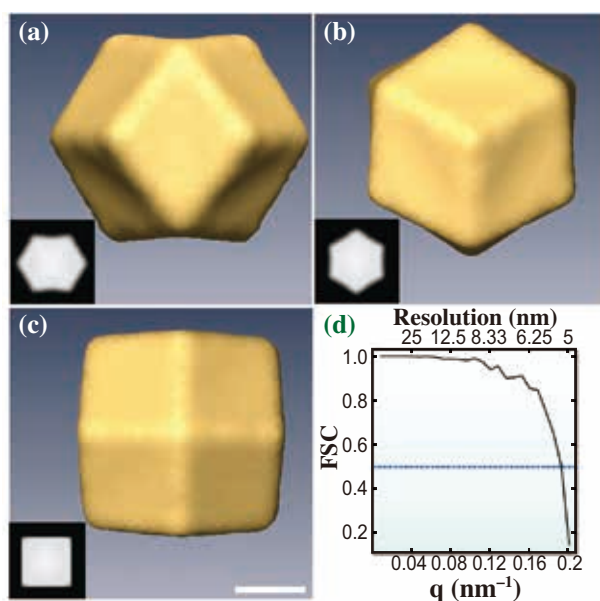


Fig. 2. 3D reconstruction of the single-shot diffraction pattern. (a-c) Iso-surface renderings of the final 3D reconstruction along the 2-, 3- and 4-fold rotational symmetry, respectively. The insets show 3.3-nm-thick center-slices of the 3D reconstruction along the corresponding directions. The variation of the electron density inside the nanocrystal is within $\pm 10\%$, which is mainly due to noise in the single-shot diffraction pattern. (d) Fourier shell correction (FSC) comparison between two independently reconstructed gold nanocrystals. Based on the FSC = 0.5 criterion, a 3D resolution of the reconstructions was estimated to be ~ 5.5 nm.

diffraction patterns was performed, implementing the 3D oversampling smoothness (OSS) algorithm with an additional constraint that incorporates symmetry into the reconstruction [4]. Figures 2(a-c) show the iso-surface renderings along two-, three- and four-fold rotational symmetry of the reconstructed object. The insets in Figs 2(a-c) are central slices perpendicular to each symmetry axis. After determining the relative orientations and aligning the diffraction intensity of six selected high quality diffraction patterns, we assembled a 3D diffraction pattern from 288 symmetrized patterns. Using OSS with symmetry, the average 3D structure of gold nanocrystals was reconstructed. We estimated the 3D resolution of these reconstructions to be 5.5 nm, which is currently limited by the detector geometry arrangement.

To confirm these results, the reconstructed images were compared with the calculated model of trisoctahedral nanocrystals. For trisoctahedron with {661} exposure facets, facet angles α , β and γ shown in Fig. 3(a) are its intrinsic feature, measuring 119.0° , 113.8° and 166.6° respectively. We measured all these parameters for each of the reconstructed particles.

Our results indicated that the overall shape and size of these nanocrystals are in good agreement with expected values.

As symmetry exists in many nanocrystals and virus, this method provides a promising way to analyze these particles at nanometer resolution on femtosecond timescales. By recording enough diffraction patterns from identical samples, the 3D reconstruction method can also be used to reconstruct the true 3D structure of weakly scattering objects.

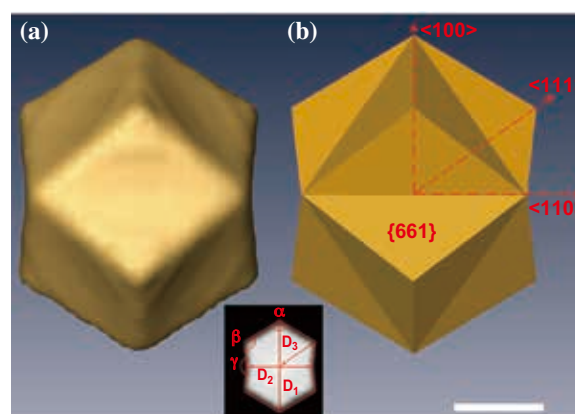


Fig. 3. 3D model of a trisoctahedral gold nanocrystal constructed from a single-shot 3D reconstruction. (a) Iso-surface rendering and 3.3-nm-thick central slice (inset) of the nanocrystal along the 2-fold rotational symmetry, of which D1, D2, D3, α , β and γ are six parameters used to characterize the shape, size and facets of the reconstructed nanocrystal. (b) 3D model of the concave trisoctahedral nanocrystal constructed from (a), containing exposed high index {661} facets.

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References

- [1] J. Miao *et al.*: Nature **400** (1999) 342-344.
- [2] R. Xu, H. Jiang, C. Song, J.A. Rodriguez, Z. Huang, C.-C. Chen, D. Nam, J. Park, M. Gallagher-Jones, S. Kim, S. Kim, A. Suzuki, Y. Takayama, T. Oroguchi, Y. Takahashi, Jiadong Fan, Y. Zou, T. Hatsui, Y. Inubushi, T. Kameshima, K. Yonekura, K. Tono, T. Togashi, T. Sato, M. Yamamoto, M. Nakasako, M. Yabashi, T. Ishikawa and J. Miao: Nat. Commun. **5** (2014) 4061.
- [3] T. Ishikawa *et al.*: Nat. Photonics **6** (2012) 540.
- [4] J.A. Rodriguez *et al.*: J. Appl. Cryst. **46** (2013) 312.

Serial femtosecond rotation crystallography: an opportunity for high-resolution crystal structure determination free from radiation damage

Radiation damage from X-ray irradiation has been a longstanding problem in protein crystallography. X-ray crystallographic analysis is very powerful method to determine the high-resolution atomic coordinates of proteins. The resultant atomic coordinates are very informative to understand the structural basis of protein function. Nevertheless, in some cases a high-resolution crystal structure does not necessarily reflect the nature of a protein due to the radiation damage. However, radiation damage of proteins is probably unavoidable as long as conventional light sources such as synchrotron (SR) are used because the normal exposure time to acquire a diffraction image (e.g., several seconds) is long enough for X-ray dependent formation of reactive molecules and successive attacks on protein molecule in a crystal. Despite the temperature dependency of the diffusion process for a reactive molecule, a cryogenic temperature is insufficient to prevent radiation damage during data collection. A crystallographic method that is free of radiation damage and provides a high spatial resolution has been desired.

X-ray free electron laser (XFEL) opens the door for the structural biology based on high-resolution crystal structure analysis free of radiation damage. The concept to prevent radiation damage is femtosecond data collection. An X-ray pulse of SACLA contains middle of 10^{10} photons for a pulse duration less than 10 femtoseconds, which makes it possible to acquire a diffraction image 100 times faster than the X-ray

dependent formation of reactive molecules. Actually, the total pulse flux is almost equal to the flux available by a one second exposure time at beamline **BL32XU** as an advanced microbeam beamline for protein crystallography.

To utilize the beam feature of SACLA in high-resolution protein crystal structure analysis without radiation damage, a new diffractometer was installed at beamline **BL3 EH3** of SACLA [1] (Fig. 1). The concept of the camera with a goniometer is combined usage of a brilliant femtosecond X-ray pulse and large crystals compatible with conventional protein crystallography using SR in size. The large diffraction volume in a large crystal strengthens the diffraction intensity of a Bragg spot, resulting in an improved diffraction resolution. In addition, we can utilize the knowhow that is accumulated in the long history of protein crystallography to grow high quality large crystals. To date, we have successfully determined the high-resolution crystal structure of very large macromolecular complexes without radiation damage: bovine heart cytochrome *c* oxidase at a 1.90-Å resolution [1] and cyanobacteria photosystem II at a 1.95-Å resolution [2]. Those are outstanding resolutions compared to the resolution of the structure determination of proteins with similar molecular weights by serial femtosecond crystallography using micrometer crystals.

Data collection from large crystals is performed by a step scan in which the diffraction intensity of a Bragg spot with a certain spread is sampled by few

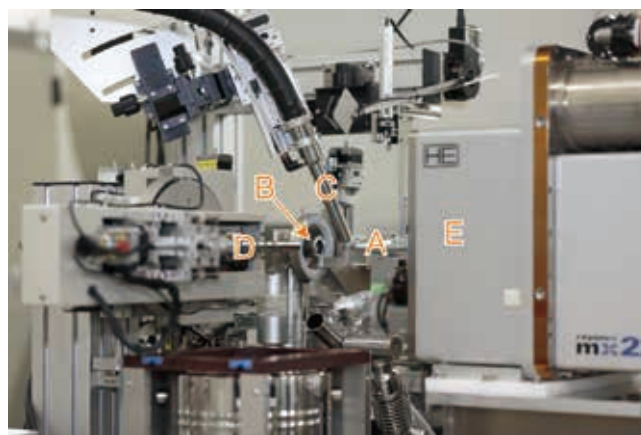


Fig. 1. Close up of the sample position of the diffractometer installed at EH3/BL3 of SACLA. (A) Goniometer to mount a large crystal, (B) X-ray coaxial camera for crystal alignment. X-ray pulse passes through a pinhole at the center of the objective lens. (C) Cryostat. (D) Crystal exchange robot SPACE. (E) CCD X-ray detector.

still diffraction images that are separated by a step angle of $\delta\theta$ (Fig. 2(a)). The data collection sequence is as follows. Acquire a still diffraction image at a crystal rotation angle of θ . Rotate the crystal by a step angle of $\delta\theta$. Acquire the next diffraction image at a crystal rotation angle of $\theta + \delta\theta$. An appropriate step angle is an angle smaller than one-third of the crystal mosaicity. A large step angle increases the unobserved diffraction intensities, resulting in decay of the quality of diffraction intensity data. The preliminary experiment performed at BL32XU shows that the refined structures are comparable between the data collected by the step angle of half of crystal mosaicity and the data collected by the conventional oscillation method [1].

As shown above, in this method, a series of still diffraction images are collected from a single crystal. The X-ray pulse breaks the portion of crystal irradiated after producing a diffraction image without radiation damage [3]. Thus, an appropriate distance between adjacent paths of X-ray pulses in a crystal guarantees radiation damage-free diffraction intensity data (Fig. 2(b)). In actual measurements at SACLA, an irradiation of X-ray pulse decreased the order of crystal lattice in the region surrounding the beam path with approximately 11 μm radius [1].

The 50- μm separation of the path of the X-ray pulses in a crystal is effective to avoid radiation damage not only on the large scale but also on the small scale such as structural change in the protein. In the case of the structure determination of bovine heart cytochrome *c* oxidase bound a peroxide ligand, the X-ray dependent photolysis of the peroxide ligand is not observed, although it has been a longstanding problem in SR experiments [1,4]. The bond length between the peroxide oxygen atoms is refined to be 1.55 Å, which is acceptable for a bond length of peroxide ligand. In contrast, the bond length has not been shortened below 1.7 Å with SR data due to contamination of the photolysis product of the peroxide ligand. In addition, the peak height of the electron density next to the side chain of the tyrosine residue in the ligand binding site, which increases concomitantly with the photolysis of the peroxide ligand, is kept as low as the basal level observed in the cytochrome *c* oxidase whose peroxide ligand is substituted by a cyanide ion.

This method, which can be called as serial femtosecond rotation crystallography, provides a practical way to determine the high resolution crystal structure of huge biological macromolecules without radiation damage.

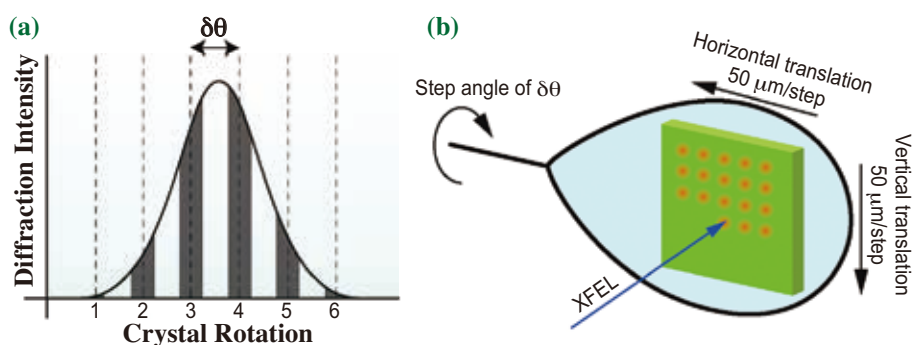


Fig. 2. Schematic drawing of the data collection procedure. (a) Diffraction intensity of a Bragg spot is sampled by a set of discrete still diffraction images. Total diffraction intensity is estimated based on the observed partial intensities colored in gray. (b) Data collection by crystal translation and rotation. Crystal is translated by 50 μm to acquire a diffraction image from the portion of the crystal that is unaffected by previous X-ray irradiations. In addition, the crystal is rotated by a step angle of $\delta\theta$ to sample the diffraction intensity of a Bragg spot with a certain spread of a few diffraction images.

Hideo Ago

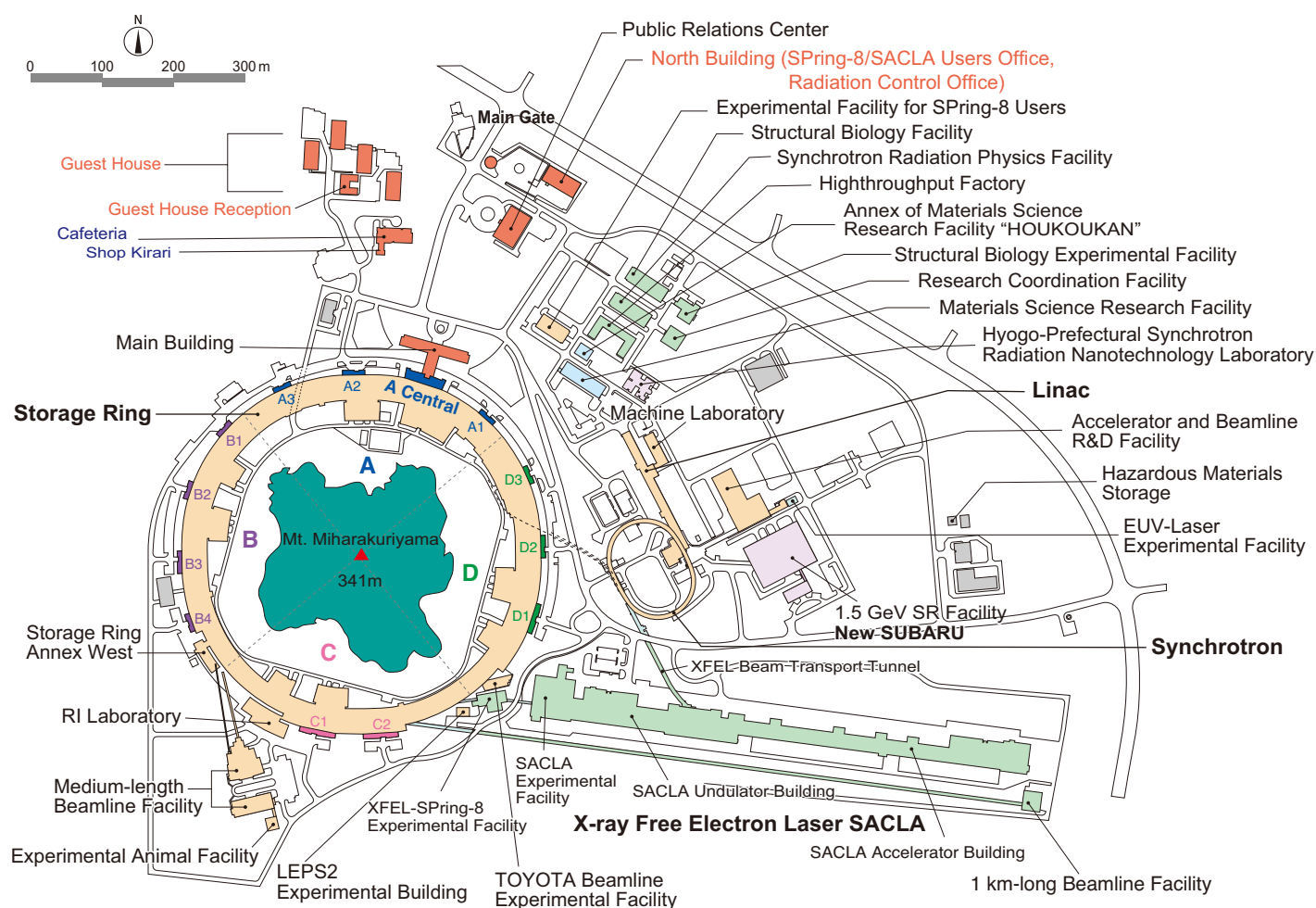
RIKEN SPring-8 Center

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References

- [1] K. Hirata, K. Shinzawa-Itoh, N. Yano, S. Takemura, K. Kato, M. Hatanaka, K. Muramoto, T. Kawahara, T. Tsukihara, E. Yamashita, K. Tono, G. Ueno, T. Hikima, H. Murakami, Y. Inubushi, M. Yabashi, T. Ishikawa, M. Yamamoto, T. Ogura, H. Sugimoto, J.-R. Shen, S. Yoshikawa and H. Ago: *Nat. Methods* **11** (2014) 734.
- [2] M. Suga *et al.*: *Nature* **517** (2015) 99.
- [3] R. Neutze *et al.*: *Nature* **406** (2000) 752.
- [4] H. Aoyama *et al.*: *Proc. Natl. Acad. Sci. USA* **106** (2009) 2165.

FACILITY STATUS



SPring-8

I. Introduction

As described in the next section in detail, SPring-8 was stably operated throughout 2014 with the total operation time of the storage ring and the total user beam time of 5081 h and 4058 h, respectively. The total downtime amounted to 17 h, which is less than 0.42% of the total operation time, giving almost 100% beamtime availability to users. The total number of proposals submitted in 2014 reached 1904, among which 1454 were accepted.

Regarding the SPring-8 utilization system, there were three improvements implemented. First, it has become compulsory for proposals of industrial applications to include, at least, an investigator who is employed by a private company or related institution. This is to further facilitate scientific cooperation between industry and academia. Second, the public beamline portfolio for macromolecular crystallography, consisting of BL41XU, BL38B1, BL32XU, BL26B1 and BL26B2, has become more efficient by introducing a flexible beamtime allocation management system. The improved beamline portfolio is expected to efficiently and effectively meet the beamtime requests recently increasing in this field. Third, a new proposal category called the Social

Interest Program has started calling for proposals. The aim of the program is to proactively support those users joining SPring-8 from archaeology, paleontology, space science, and so forth, to the outcome from which public in general has a high-level of interest.

Concerning the contract beamlines, there were two interim reviews conducted for “BL03XU Advanced Softmaterial (Advanced Softmaterial Beamline Consortium)” and “BL36XU Catalytic Reaction Dynamics for Fuel Cells (The University of Electro-Communications),” both of which were authorized to continue their projects. Upon the expiration of their contract terms, review were also conducted for “BL33LEP Laser-Electron Photon (Research Center for Nuclear Physics, Osaka University)” and “BL08B2 Hyogo BM (Hyogo Prefecture),” and their proposals for the next terms were both approved.

Over the course of the year, various types of scientific meetings were held, such as the SPring-8 Symposium, SPring-8 Conference, and The Joint Conference on Industrial Applications of SPring-8, in order to keep active dialogues between SPring-8 and its users community, SPRUC.



II. Machine Operation

The operation statistics for the last five fiscal years are shown in Fig. 1. In the FY2014, total operation time of the accelerator complex was 5099.3 h. The operation time of the storage ring was 5080.7 h, 79.9% of which (4057.8 h) was available for SR experiments. The downtime resulting from failure accounted for 0.42% (17.1 h) of the total user time, and no great loss of user time exceeding several hours occurred. Although the operation time for FY2013 was about 800 h less than that for a typical fiscal year due to the large-scale renovations of the aging cooling water and air-conditioning systems, that for FY2014 was restored to the average.

A variety of operation modes is one of the characteristics of SPRING-8. The operation modes are grouped into the multi-bunch mode, several-bunch mode, and hybrid-filling mode. There has been no user operation with the multi-bunch mode since FY2011. The several-bunch mode consists of equally spaced bunches or bunch-trains, i.e., 203 bunches, or 29 trains of 11 bunches, and the hybrid-filling mode is composed of a long bunch train and isolated single bunches, as shown in Table 1, where the shares of user time of the operation modes are also shown. The new operation mode of

11/29-filling + 1 single bunch, which started user operation in 2012, plays an important role. A bunch impurity of less than 10^{-8} is routinely maintained in the top-up operation. Table 2 summarizes the useful beam parameters of the storage ring.

Table 1. Beam parameters of SPRING-8 storage ring

Energy [GeV]	8
Number of buckets	2436
Tunes (ν_x/ν_y)	41.14 / 19.34
Current [mA]:	
single-bunch	12
multi-bunch	100
Bunch length (σ) [psec]	13
Horizontal emittance [nm·rad]	2.4 *
Vertical emittance [pm·rad]	4.8 *
Coupling [%]	0.2
RF Voltage [MV]	16
Momentum acceptance [%]	3.2 (~256 MeV)
Beam size (σ_x/σ_y) * [μ m]	
Long ID section	333 / 7
ID section	316 / 5
BM1 section	94 / 12
BM2 section	100 / 12
Beam divergence (σ'_x/σ'_y) * [μ rad]	
Long ID section	8 / 0.7
ID section	9 / 1.0
BM1 section	58 / 0.5
BM2 section	68 / 0.5
Operational chromaticities (ξ_x/ξ_y)	+2 / +2 **
Lifetime [h]:	
100 mA (multi-bunch)	~ 250
1 mA (single-bunch)	~ 30
Horizontal dispersion [m]:	
Long ID section	0.153
ID section	0.146
BM1 section	0.039
BM2 section	0.059
Fast orbit stability (0.1 – 200 Hz) [μ m]:	
horizontal (rms)	~ 4
vertical (rms)	~ 1

* Assuming 0.2% coupling
** With bunch-by-bunch feedback

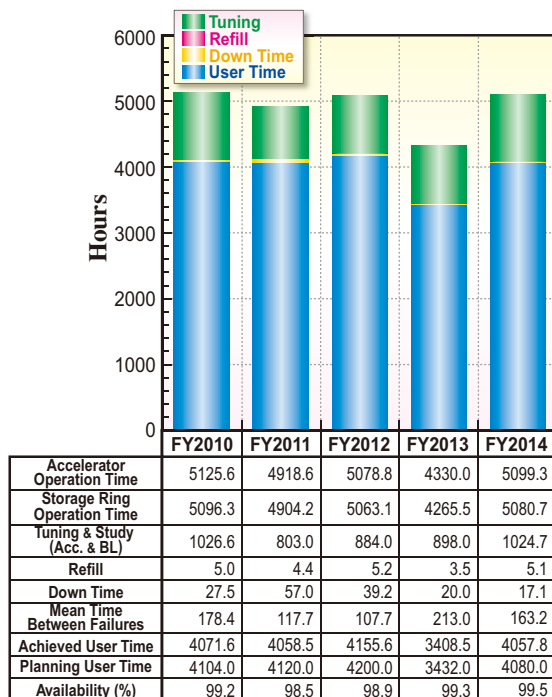


Fig. 1. Operation statistics for most recent five fiscal years.

Table 2. Filling patterns in FY2014

	Single bunch current (mA)	Share of operation time
203 bunches		22.4%
11 bunch-train × 29		31.8%
1/7-filling + 5 single bunches	3 mA	10.0%
2/29-filling + 26 single bunches	1.4 mA	0.0%
1/14-filling + 12 single bunches	1.6 mA	12.4%
4/58-filling + 53 single bunches	1 mA	2.9%
11/29-filling + 1 single bunch	5 mA	20.6%

III. Beamlines

The SPring-8 storage ring can accommodate up to 62 beamlines: 34 insertion devices, 4 long undulators, and 24 bending magnets. At the time of writing, 56 beamlines are in operation, covering a wide variety of research fields involving synchrotron radiation science and technology. The beamlines are classified into the following four types.

- (1) Public Beamlines
- (2) Contract Beamlines
- (3) RIKEN Beamlines
- (4) Accelerator Diagnostics Beamlines

There are now 26 public beamlines in full operation. The beamlines, that are proposed and constructed by external organizations, such as universities, research institutes, and private companies, are

called contract beamlines and are exclusively used by the contractors for their own research purposes. At present, 19 contract beamlines are in operation. The contract beamlines include the NSRRC BM (BL12B2) and NSRRC ID (BL12XU) beamlines, which were constructed by the National Synchrotron Radiation Research Center of Taiwan. The beamlines constructed by RIKEN are called RIKEN beamlines, which are mainly used for RIKEN's own research activities and are partly available for public use. RIKEN is now operating 9 beamlines and is reconstructing one beamline. In addition, two accelerator diagnostics beamlines are in operation.

To illustrate the beamline portfolio of SPring-8, a beamline map is shown in Fig. 2 together with the beamline classification. The research fields of each beamline are presented in Table 3.

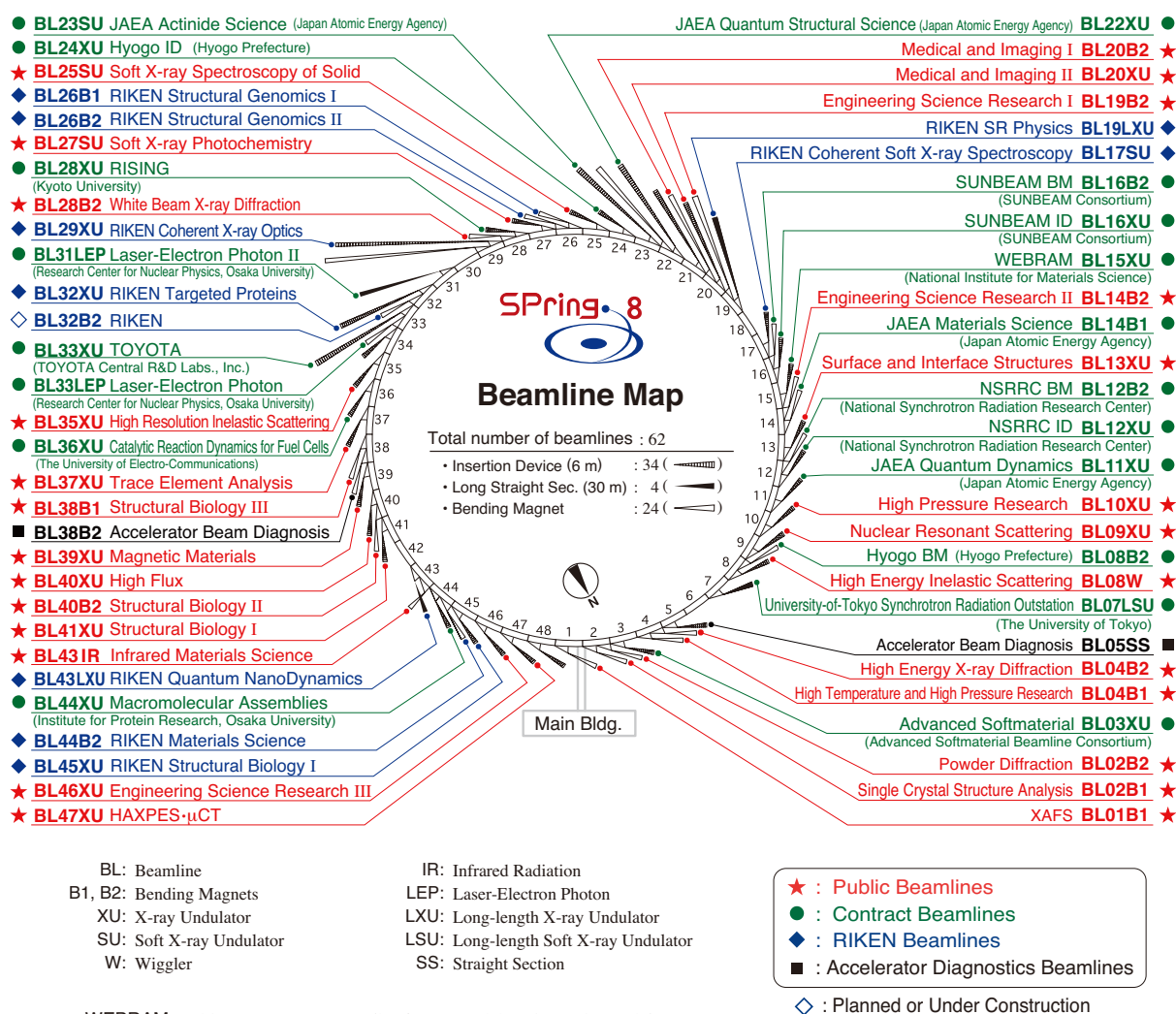


Fig. 2. Beamline map.

Table 3. List of beamlines

BL #	Beamline Name	(Public Use) or (First Beam)	Areas of Research
★ Public Beamlines			
BL01B1	XAFS	(Oct. 1997)	XAFS in wide energy region (3.8 to 113 keV). XAFS of dilute systems and thin films. Quick XAFS with a time resolution of seconds to as tenth seconds.
BL02B1	Single Crystal Structure Analysis	(Oct. 1997)	Charge density study and crystal structure analysis from accurate single crystal diffraction measurements. (X-ray energy range: 8 – 115 keV)
BL02B2	Powder Diffraction	(Sept. 1999)	Charge density study and phase identification of crystalline materials from accurate powder diffraction measurements. (X-ray energy range: 12.4 – 35 keV)
BL04B1	High Temperature and High Pressure Research	(Oct. 1997)	High temperature and high pressure research with the multi-anvil press by powder X-ray diffraction, radiography and ultrasonic measurement.
BL04B2	High Energy X-ray Diffraction	(Sept. 1999)	Structural biology. Macromolecular crystallography. Microcrystallography. High resolution data collection.
BL08W	High Energy Inelastic Scattering	(Oct. 1997)	Magnetic Compton scattering. High-resolution Compton scattering. High-energy Bragg scattering. High-energy fluorescent X-ray analysis.
BL09XU	Nuclear Resonant Scattering	(Oct. 1997)	Lattice dynamics using nuclear inelastic scattering. Mössbauer spectroscopy, especially for the surface/interface study and under the extreme conditions.
BL10XU	High Pressure Research	(Oct. 1997)	Structure analysis and phase transitions under ultra high pressure (DAC experiment). Earth and planetary science.
BL13XU	Surface and Interface Structures	(Sept. 2001)	Atomic-scale structural analysis of surfaces and interfaces of crystalline materials, ultra-thin films, and nanostructures. Surface X-ray diffraction (SXRD). Microbeam diffraction.
BL14B2	Engineering Science Research II	(Sept. 2007)	XAFS in wide energy region (3.8 to 72 keV). XAFS of dilute systems and thin films.
BL19B2	Engineering Science Research I	(Nov. 2001)	Residual stress measurement. Structural analysis of thin film, surface, interface. Powder diffraction. X-ray imaging, X-ray topography. Ultra-small angle X-ray scattering.
BL20XU	Medical and Imaging II	(Sept. 2001)	Microimaging. Hard X-ray microbeam/scanning microscopy, imaging microscopy, microtomography, phase-contrast microtomography with Bonse-Hart interferometer, X-ray holography, coherent X-ray optics, and other experiments on X-ray optics and developments of optical elements. Medical application. Microangiography, refraction-enhanced imaging, radiation therapy, phase-contrast CT using interferometer. Ultra-small angle scattering.
BL20B2	Medical and Imaging I	(Sept. 1999)	Microimaging: microtomography, phase-contrast microtomography with Bonse-Hart interferometer and grating interferometer for biological specimen and other kinds of specimen. Evaluation and development of various kinds of optical elements for novel imaging techniques. Large field X-ray topography.
BL25SU	Soft X-ray Spectroscopy of Solid	(Apr. 1998)	Study of electronic state of solids by soft X-ray photoemission spectroscopy (PES) including angle-resolved PES (ARPES). Atomic arrangement analysis of surfaces by photoelectron diffraction (PED) technique using two-dimensional photoemission analyzer. Magnetic state analysis by magnetic circular dichroism (MCD) of soft X-ray absorption and its element-specific magnetization curve measurements. Chemical and magnetic imaging by soft X-ray scanning microscopy and photoelectron emission microscopy (PEEM).
BL27SU	Soft X-ray Photochemistry	(May 1998)	Ambient atmospheric pressure. Soft X-ray photoabsorption spectroscopy. Chemical state analysis of light elements in dilute samples (NEXAFS). High resolution atomic and molecular electron spectroscopy. Dissociation dynamics of inner-shell excited molecules. (Molecular science) Photoemission and soft-X-ray emission spectroscopy for solids. (Solid state and surface physics)
BL28B2	White Beam X-ray Diffraction	(Sept. 1999)	White X-ray diffraction and topography. Time-resolved energy-dispersive XAFS (DXAFS) for studies of chemical and/or physical reaction process. Biomedical imaging and radiation biology studies.
BL35XU	High Resolution Inelastic Scattering	(Sept. 2001)	Materials dynamics on ~meV energy scales using inelastic X-ray scattering (IXS).
BL37XU	Trace Element Analysis	(Nov. 2002)	X-ray microbeam spectrochemical analysis. Ultra trace element analysis. High energy X-ray fluorescence analysis.
BL38B1	Structural Biology III	(Oct. 2000)	Structural biology. Macromolecular crystallography. Automatic data collection.
BL39XU	Magnetic Materials	(Oct. 1997)	X-ray magnetic circular dichroism (XMCD) spectroscopy and element-specific magnetometry under multiple-extreme conditions. XMCD/XAS using a sub-micron X-ray beam. X-ray emission spectroscopy. Resonant X-ray magnetic scattering.
BL40XU	High Flux	(Apr. 2000)	Time-resolved diffraction and scattering experiments. Microbeam X-ray diffraction and scattering experiments. X-ray photon correlation spectroscopy. Fluorescence analysis. Quick XAFS. Submicrometer-scale single crystal structure analysis with high flux and zone plate focused X-ray beam. Single shot imaging with X-ray choppers. Laser pump-X-ray probe experiment.
BL40B2	Structural Biology II	(Sept. 1999)	Noncrystalline small and wide angle X-ray scattering.
BL41XU	Structural Biology I	(Oct. 1997)	Structural biology. Macromolecular crystallography. Microcrystallography. High resolution data collection.
BL43IR	Infrared Materials Science	(Apr. 2000)	Infrared microspectroscopy.
BL46XU	Engineering Science Research III	(Nov. 2000)	Structural characterization of thin films by X-ray diffraction and X-ray reflectivity measurement. Residual stress measurement. Time resolved X-ray diffraction measurement. Hard X-ray photoemission spectroscopy.
BL47XU	HAXPES-μCT	(Oct. 1997)	Hard X-ray photoelectron spectroscopy (HAXPES). Depth analysis of angle resolved HAXPES with wide acceptance lens. Projection type microtomography. Imaging type microtomography. Hard X-ray microbeam/scanning microscopy.

BL #	Beamline Name	(Public Use) or (First Beam)	Areas of Research
● Contract Beamlines			
BL03XU	Advanced Softmaterial (Advanced Softmaterial Beamline Consortium)	(Nov. 2009)	Structural characterization of softmaterials using small- and wide-angle X-ray scattering. Grazing-incidence small- and wide-angle X-ray scattering for thin films. X-ray diffraction and reflectivity measurements for softmaterials.
BL07LSU	University-of-Tokyo Synchrotron Radiation Outstation (The University of Tokyo)	(Oct. 2009)	Time-resolved soft X-ray spectroscopy, nano-beam photoemission spectroscopy, ultra high-resolution soft X-ray emission spectroscopy, and any methods requiring the highly brilliant soft X-ray beam.
BL08B2	Hyogo BM (Hyogo Prefecture)	(Jun. 2005)	XAFS in a wide energy region. Small angle X-ray scattering for structural analyses of polymer and nanocomposite materials. X-ray topography. Imaging. Powder diffraction with a high angular-resolution.
BL11XU	JAEA Quantum Dynamics	(Oct. 1998)	Nuclear scattering. Surface and interface structure analysis with MBE. Inelastic X-ray scattering. XAFS.
BL12B2	NSRRC BM (National Synchrotron Rad. Res. Center)	(Oct. 2000)	X-ray absorption spectroscopy. Powder X-ray diffraction. High resolution X-ray scattering. Protein crystallography.
BL12XU	NSRRC ID (National Synchrotron Rad. Res. Center, Taiwan)	(Dec. 2001)	High resolution non-resonant or resonant inelastic X-ray scattering. High resolution near-edge X-ray Raman scattering. Phase transitions under high-pressure, low and high temperatures. High-resolution X-ray absorption and emission spectroscopy. X-ray physics and optics.
BL14B1	JAEA Materials Science	(Dec. 1997)	Materials science under high-temperature and high-pressure. <i>In situ</i> study on catalysis using dispersive XAFS. X-ray diffraction for structure physics.
BL15XU	WEBRAM (National Institute for Materials Science)	(Jan. 2000)	Hard X-ray photoelectron spectroscopy. High-precision X-ray powder diffraction.
BL16B2	SUNBEAM BM (SUNBEAM Consortium)	(Oct. 1998)	Characterization of secondary battery related materials, semiconductors, fuel cells, catalysts, and several industrial materials with using X-ray absorption fine structure measurements, X-ray diffraction (including X-ray reflectivity technique) and X-ray topography.
BL16XU	SUNBEAM ID (SUNBEAM Consortium)	(Oct. 1998)	Characterization of semiconductor materials, secondary batteries, fuel cells, catalysts, electrical display related materials, and structural materials with using X-ray diffraction, X-ray microbeam based evaluation technique (including X-ray magnetic circular dichroism), and fluorescence X-ray analysis.
BL22XU	JAEA Quantum Structural Science	(May 2002)	Materials science under high-pressure. Resonant X-ray scattering. Speckle scattering. Residual stress/strain distribution analysis.
BL23SU	JAEA Actinide Science	(Feb. 1998)	Surface chemistry with supersonic molecular beam. Biophysical spectroscopy. Photoelectron spectroscopy. Magnetic circular dichroism.
BL24XU	Hyogo ID (Hyogo Prefecture)	(May. 1998)	Surface/interface analysis for industry by fluorescent X-ray analysis, strain measurements and grazing incidence X-ray diffraction. Microbeam formation studies for materials and life sciences. Micro-SAXS for local long-range structure analysis.
BL28XU	RISING (Kyoto University) RISING: Research & Development Initiative for Scientific Innovation of New Generation Batteries	(Apr. 2012)	Analysis of rechargeable batteries. Time-resolved X-ray diffraction and XAFS with microbeam. XAFS of dilute systems and thin films. Dispersive XAFS. Hard X-ray photoelectron spectroscopy.
BL31LEP	Laser-Electron Photon II (RCNP, Osaka University)	(Oct. 2013)	Production of high intensity GeV photon beam by laser-backward Compton scattering. Hadron physics via photonuclear and photoneuclear reactions. Test and calibration of detectors with GeV gamma-ray and converted electrons/positrons.
BL33LEP	Laser-Electron Photon (RCNP, Osaka University)	(Jun. 1999)	Meson photoproduction from nucleon and nucleus. Photoexcitation of hyperons, nucleon resonances, and other exotic states. Photonuclear reactions. Beam diagnoses. Test and calibration of detectors with GeV photon beam.
BL33XU	TOYOTA (TOYOTA Central R&D Labs., Inc.)	(Apr. 2009)	Time-resolved XAFS. Characterization of industrial materials, such as catalysts, secondary batteries, fuel cells.
BL36XU	Catalytic Reaction Dynamics for Fuel Cells (The University of Electro-Communications)	(Jan.2013)	Real time analysis of catalytic reaction dynamics for fuel cells: time resolved XAFS, 2D spatial resolved XAFS, depth resolved XAFS, 3D laminography XAFS, ambient pressure hard X-ray photoelectron spectroscopy.
BL44XU	Macromolecular Assemblies (IPR, Osaka University)	(May 1999)	Crystal structure analysis of biological macromolecular assemblies (e.g., membrane complexes, protein complexes, protein-nucleic acid complexes, and viruses).
◆ RIKEN Beamlines			
BL17SU	RIKEN Coherent Soft X-ray Spectroscopy	(Sept. 2003)	High resolution photoemission spectroscopy. Soft X-ray emission spectroscopy for liquid and biological samples. Soft X-ray diffraction spectroscopy. Surface science.
BL19LXU	RIKEN SR Physics	(Oct. 2000)	SR science with highly brilliant X-ray beam.
BL26B1	RIKEN Structural Genomics I	(Apr. 2002)	Structural genomics research based on single crystal X-ray diffraction.
BL26B2	RIKEN Structural Genomics II	(Apr. 2002)	Structural genomics research based on single crystal X-ray diffraction.
BL29XU	RIKEN Coherent X-ray Optics	(Dec. 1998)	X-ray optics, especially coherent X-ray optics.
BL32XU	RIKEN Targeted Proteins	(Oct. 2009)	Protein microcrystallography.
BL43LXU	RIKEN Quantum NanoDynamics	(Oct. 2011)	High resolution inelastic X-ray scattering for investigating atomic and electronic dynamics.
BL44B2	RIKEN Materials Science	(Feb. 1998)	Structural materials science research using powder X-ray diffraction.
BL45XU	RIKEN Structural Biology I	(Jul. 1997)	Time-resolved and static structures of non-crystalline biological materials using small-angle scattering and diffraction techniques.
■ Accelerator Diagnostics Beamlines			
BL05SS	Accelerator Beam Diagnosis	(Mar. 2004)	Accelerator beam diagnostics. R&D of accelerator components. Nano-forensic science.
BL38B2	Accelerator Beam Diagnosis	(Sept. 1999)	Accelerator beam diagnostics.

IV. User Program and Statistics

SPRING-8 calls for public use proposals twice a year in principle. The submitted proposals are reviewed by the SPRING-8 Proposal Review Committee (SPRING-8 PRC). Since 1997, SPRING-8 has accepted a variety of proposals. For the promotion of research on industrial applications at SPRING-8, the Industrial Application Division was established in 2005. With consultation support for industrial users given by the division's coordinators, currently, Industrial Application Proposals account for approximately 20% of the total number of proposals conducted at the public beamlines. In addition, the Measurement Service was introduced in 2007B, in which the personnel of the Industrial Application Division carry out XAFS measurements on behalf of users at BL14B2. SPRING-8 also launched a Protein Crystallography Data Collection Service at BL38B1 and Powder X-ray

Diffraction Measurement Service at BL19B2 in 2009B, a Hard X-ray Photoemission Spectroscopy (HAXPES) Measurement Service, and a Thin Film Analysis (GIXD/XRR) Measurement Service at BL46XU in 2012B, and a Small Angle Scattering (SAXS) Measurement Service at BL19B2 in 2014B.

SPRING-8 has consistently provided ~4000 h of user beamtime per year. Since the start of operation in 1997, SPRING-8 has succeeded in providing users with a total beamtime of 66,763 h. The beamtime available to the users, the number of experiments conducted, and the number of user visits at the public and contract beamlines are summarized in Fig. 3. Part of the proposals are for proprietary use, for which refereed reports are not required.

More details can be found at http://www.spring8.or.jp/en/about_us/spring8data/.

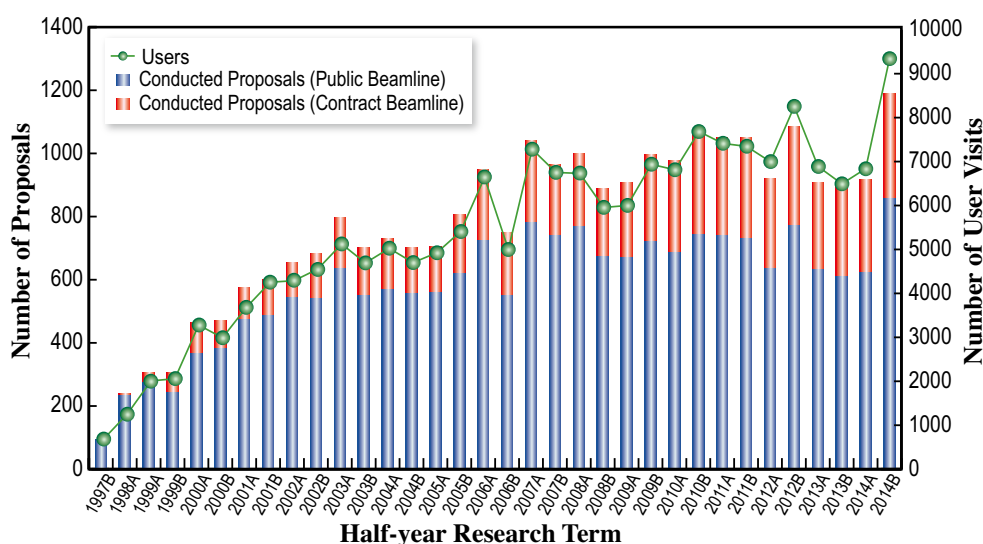


Fig. 3. Numbers of user visits and conducted experiments.

V. Research Outcomes

As of March 2015, the total number of registered refereed papers since the opening of SPRING-8 is 10,468, out of which 8,692 papers resulted from the use of public beamlines (including public use at other beamlines), 2,083 papers from that of contract beamlines, 1,115 papers resulted from the use of RIKEN beamlines, and 497 papers resulted from hardware/software R&D. The papers resulting from the use of two or more beamlines are counted for each beamline. More details can be found at http://www.spring8.or.jp/en/about_us/spring8data/ and the publication database is available at <https://user.spring8.or.jp/uisearch/publication2/>.

VI. Budget and Personnel

When SPRING-8 started operation in 1997, it was jointly managed by RIKEN, JAERI (now JAEA), and JASRI. However, JAERI withdrew from the management of SPRING-8 on September 30, 2005. SPRING-8 is currently administered by RIKEN and JASRI in a collaborative manner.

The total budget for the operation of SPRING-8 in FY2014 was 9.324 billion yen. As of October 2014, RIKEN and JASRI have a total of 511 staff members.

VII. Research Complex

The facilities of SPing-8, SACLA, and NewsUBARU form a Center of Excellence (COE) at the SPing-8 campus, where JASRI, public beamline users, the contractors of contract beamlines, RIKEN, and the University of Hyogo work in close cooperation, forming a research complex

where each member has their own role in delivering high-quality results in the field of synchrotron radiation science and technology. The organizational charts of RIKEN and JASRI, which are at the center of this research complex, are shown in Fig. 4 and Fig. 5, respectively.

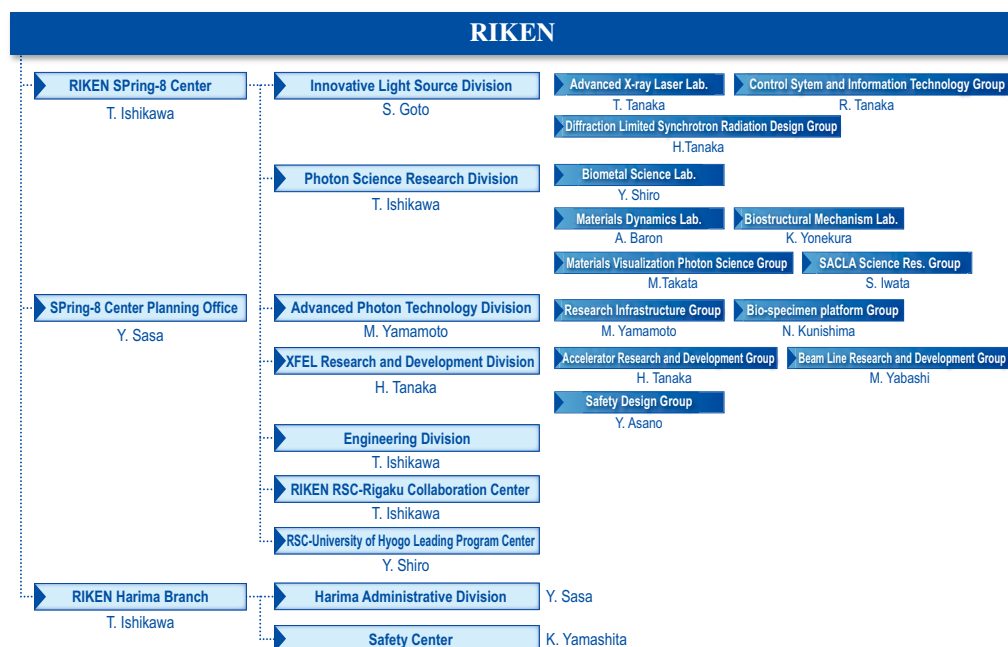


Fig. 4. RIKEN Harima Branch chart as of April 2015.

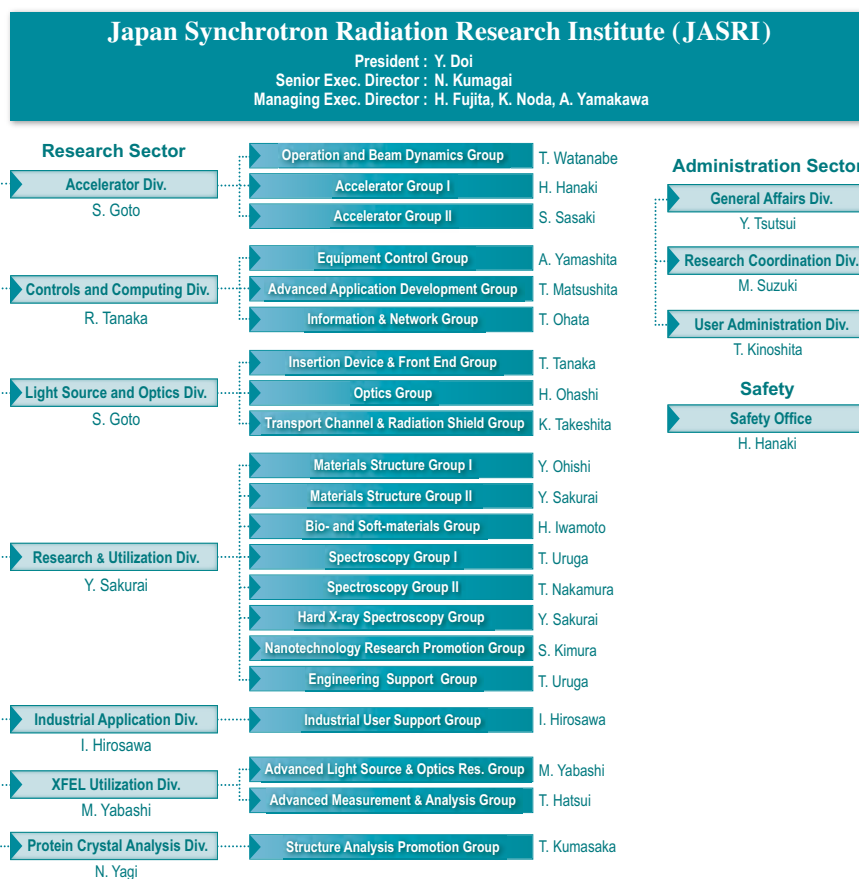


Fig. 5. JASRI chart as of April 2015.

VIII. Users Societies and Other Activities

SPRing-8 Users Community (SPRUC)

SPRing-8 Users Community (SPRUC) is a user society that includes not only all users but also potential users who are interested in using SPRing-8. In addition to these individuals, representing organizations of about 24 institutes (principal universities, national/international research institutes, industries, beamline consortiums), participate in SPRUC to discuss further promotion of the utilization of SPRing-8 from strategic and perspective viewpoints.

As one of the key activities of SPRUC, the SPRing-8 Symposium is held annually at the site of one of the representing organizations jointly with RIKEN and JASRI. The 2014 SPRing-8 Symposium, with the theme "New Dimension of Science and Technology Using SPRing-8," was held at the University of Tokyo on September 13 and 14, 2014. SPRUC provides Young Scientist Award to recognize pioneering achievements in photon science by individual young scientists using SPRing-8. The award ceremony and award lecture were held at the SPRing-8 Symposium. The SPRUC2014 Young Scientist Award was conferred to two young scientists (Dr. Ryuichi Nomura, Tokyo Institute of Technology and Dr. Hiroshi Nishimasu, The University of Tokyo). The 2015 SPRing-8 Symposium is scheduled to be held on September 13 and 14, at Kyushu University.

In accordance with the recommendation by the "WG on Reorganization of SPRUC Research Groups," SPRUC has established "SPRUC multidisciplinary research groups" that work in multidisciplinary research fields in order to promote the use of SPRing-8 in the new areas that will be important for future developments in science and technology. This was one of the main activities of SPRUC in 2014. As the beginning of this activity, four multidisciplinary research fields and four program officers leading each multidisciplinary research group were designated following comprehensive advice from the advisors of the SPRUC research groups. For the constant renewal of the system, each multidisciplinary research group works two years and is then expected to organize a new research group. The first Joint Workshop for the SPRUC Multidisciplinary Research Group was held on February 19, 2015 at the Akihabara Convention Center. The main purpose of the workshop was to exchange information between groups at the forefront of science and the advanced use of SPRing-8, thereby exploring the functions of multidisciplinary research groups in developing new research fields and discovering potential users of their groups. From 2015, the multidisciplinary research groups will undertake an innovative use of SPRing-8 through organic collaboration with JASRI and RIKEN.



*Young Scientist Award
Prof. A. Takahara, Dr. H. Nishimasu and Prof. J. Mizuki*



Other Activities

- The 22nd SPring-8 Open House
April 27, 2014 – SPring-8
- The 14th SPring-8 Summer School 2014
July 6-9, 2014 – Public Relations Center, SPring-8
- The 8th AOFSSRR School - Cheiron School 2014
September 23 - October 2, 2014 – Public Relations Center, SPring-8

Summer School



SPring-8 Open House



Cheiron School 2014



SACLA

I. Introduction

SACLA is the world's second X-ray free electron laser (XFEL), following the Linac Coherent Light Source (LCLS) in the US. Currently, these are the only two XFELs in operation. SACLA delivers the shortest wavelength XFEL. SACLA achieved its first lasing on June 7, 2011, within three months of electron beam commissioning, and launched user operations from 2012A. The user selection system for SACLA is based on that used by SPring-8. SACLA has an independent proposal review committee, the SACLA Proposal Review Committee (SACLA PRC), which reviews all received proposals.

II. Machine Operation & Beamlines

Our third year of operations proceeded without any significant issues. Operation statistics are summarized in Table 4. The ratio of downtime to user time was kept below 6.6%, a reasonably low rate for linac-based light sources.

Table 4. Operation statistics for FY2014

	Time (h)
Total operation time	6258
User time	3600
Facility tuning time	382
Study time	2276
Downtime	237 (6.6% of user time)

In 2014, two beamlines, BL3 for XFEL and BL1 for broadband spontaneous light, were open for users, while all experiments were conducted with BL3. As the newest beamline, construction of BL2 was completed during the summer shutdown, and first laser amplification was achieved on October 21.

III. User Program and Statistics

SACLA calls for public use proposals twice per year. Proposals fall into two categories: General Proposals for general research with no specific research theme, while Priority Strategy Proposals are on strategic themes that are designated by the national policy. Currently there are two strategic themes: Hierarchical Structure Dynamics of Biomolecules and Pico/Femto Second Dynamic Imaging. Table 5 shows the research themes for each group are as follows:

Table 5. Research themes for each group

1. Hierarchical Structure Dynamics of Biomolecules	1-1 Structural Analysis of Drug-targeted Membrane Protein Nanocrystals
	1-2 Imaging of Whole Cell and Its Components in the Living State
	1-3 Single Molecule Structural Analysis of Supramolecular Complex
	1-4 Dynamics Research Combining Single Molecule X-ray Diffraction Experiments and Supercomputer Analysis
	1-5 Dynamic Structural Analysis Using Pump-Probe Techniques
2. Pico/Femto Second Dynamic Imaging	2-1 Gas-Phase/Liquid-Phase/Solid-Phase Reaction Dynamics
	2-2 Ultrafast Interface Reaction Processes
	2-3 Charge Generation/Charge Transfer Dynamics
	2-4 Ultrafast Processes under Extreme Conditions
	2-5 Dynamic X-ray Spectroscopy

Table 6, Figs. 6 and 7 provide statistics on proposals, users, and beamtime. Among the proposals carried out, the number of general proposals were 13, 8, 9, 11, 8, and 12 during 2012A, 2012B, 2013A, 2013B, 2014A, and 2014B, respectively.

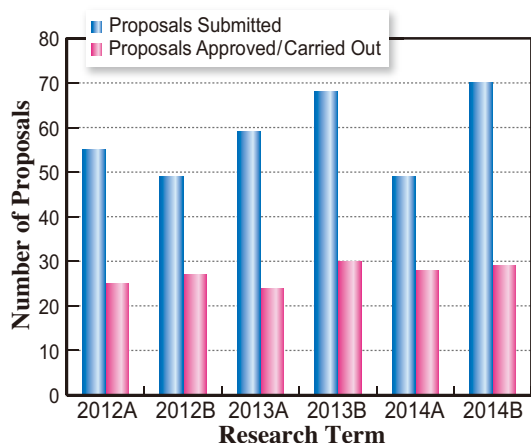


Fig. 6.

Table 6. Number of proposals submitted, proposals approved/carried out, cumulative users and beamtime available by research term

Half-year Research Term	Proposals Submitted	Proposals Approved/Carried Out	Cumulative Users	Beamtime Carried Out (Shifts)
2012A	55	25	297	126
2012B	49	27	461	154
2013A	59	24	268	117
2013B	68	30	410	139
2014A	49	28	400	147
2014B	70	29	430	140

One shift = 12 hours at SACLA beamlines

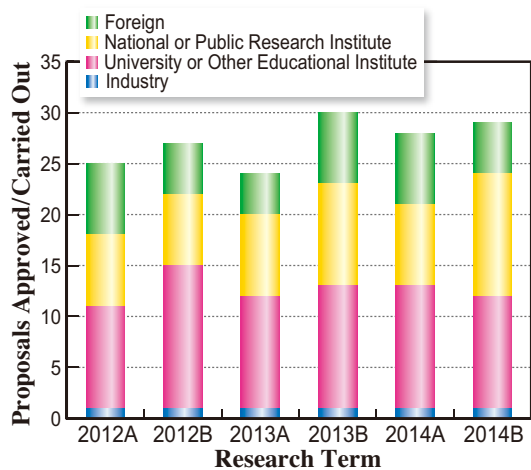


Fig. 7.

IV. Research Outcome

In 2014, 16 papers were published as user scientific activities at SACLA, while 4 papers were reported from the SACLA facility. They include 10 reports appeared in Nature-related journals and Physical Review Letters.



NewSUBARU

The NewSUBARU synchrotron light facility is operated by the Laboratory of Advanced Science and Technology for Industry (LASTI), University of Hyogo. NewSUBARU celebrated its 15th anniversary of the start of its use in 2000. This facility consists of an electron storage ring and nine beamlines.

(1) Upgraded beamlines were tuned and tested for advanced studies and industrial application. For example, a renewed spectrometer in the industrial analysis beamline BL05 started to operate for industrial use, a micro focus mirror system was tested at beamline BL07 and a new large-size mirror reflection meter started use in BL10.

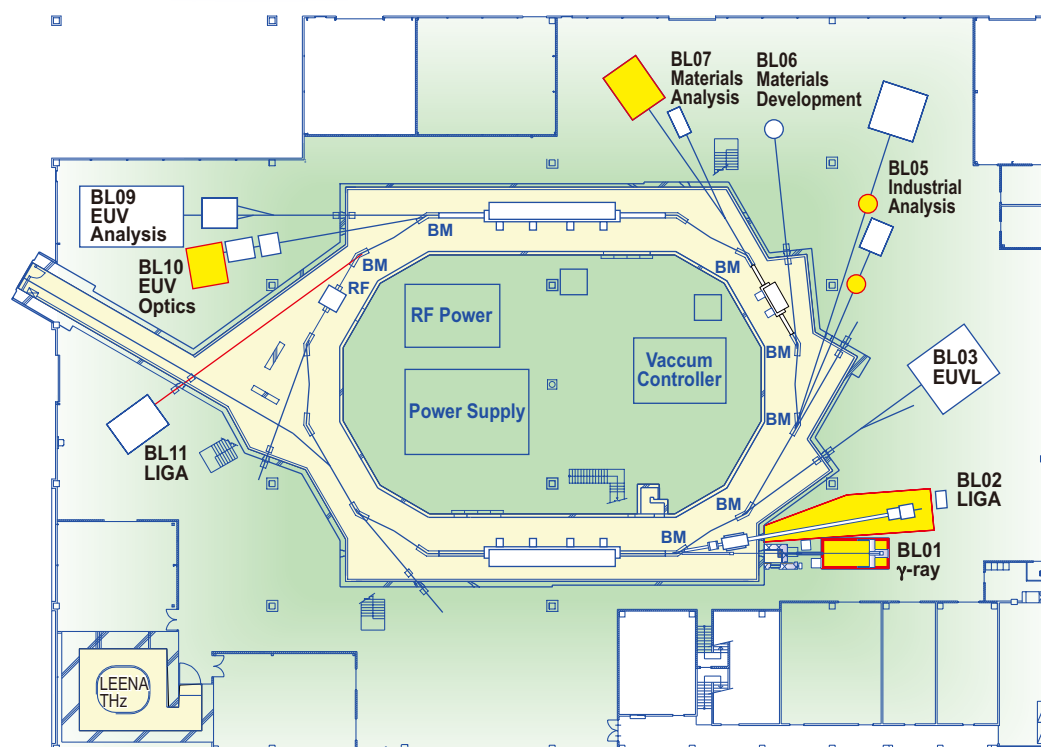
(2) The fabrication of a lab-on-a-chip device using polytetrafluoroethylene (PTFE) was achieved using the nano-micro system beamline BL02.

(3) Successful data-taking, using a polarized photon beam at the BL01 laser Compton scattering gamma-ray beamline was completed as part of international collaboration experiments. A time projection chamber (TPC) achieved the measurement of polarization in γ -ray astronomy with excellent angular precision and sensitivity in the 1-70 MeV energy range.

User use and technical assistances are supported by MEXT's "Project for Creation of Research Platforms and Sharing of Advanced Research Infrastructure".

Shuji Miyamoto

Director of LASTI, University of Hyogo



Fabrication of lab-on-a-chip by direct dry etching of PTFE using SR

The rapid detection and characterization of chemical analytes such as biological markers in tissue and fluid samples are a major challenge in medicine and environmental analysis. Recently, for the development of integrated and miniaturized microfluidic devices to achieve analysis, considerable attention has been devoted to micro-total analysis systems (μ TAS). For example, a novel μ TAS has been required to achieve the sequence of analysis operations for amino acids in extraterrestrial samples, which include extraction, filtration, hydrolysis, and evaporative drying. Precise analysis of the amino acids contained in extraterrestrial samples is very important in the study of the origin of life. We have recently had opportunities to analyze fresh extraterrestrial samples obtained by the collection of cosmic dust and samples returned from asteroids [1]. However, the amounts of the samples that can be used for analysis are limited because of their small amount. Therefore, amino acid analyses of the samples using a microfluidic chip are required.

Polytetrafluoroethylene (PTFE) has high chemical resistance and heat resistance, enabling analytical operation under severe conditions. Although PTFE has high chemical stability, its microfabrication is difficult because its small modulus of elasticity makes it unsuitable for normal mechanical processing. So far, microfluidic devices made of PTFE have not been reported. It is known that synchrotron radiation (SR) induces the scission of polymer chains of PTFE [2,3]. Figure 1 shows the mechanism of the direct dry etching of PTFE using SR. We have fabricated PTFE

microstructures by direct dry etching using SR at BL2 in NewSUBARU [4,5]. Figure 2 shows scanning electron microscope images of microfabricated PTFE plates using the SR dry etching process. As shown in Fig. 2, SR dry etching enables the microfabrication of PTFE. The combination of a PTFE microstructure fabricated using SR dry etching and compression bonding enables us to realize a PTFE microfluidic chemical analyzer.

Recently, we have developed a novel microfluidic device that had multiple microchannels and incorporated a filter structure for filtration. We also designed and manufactured novel amino acid analysis devices [5]. Figure 3 shows a schematic diagram of a fabricated amino acid analysis device. This device consists of two chips, and the function of a valve is performed by sliding the upper chip. The microchannels are incorporated into the lower chip. The lower chip has four reservoirs for reagents such as a derivatization reagent, and each reservoir can hold 10 μ l. In addition, we formed two reaction reservoirs for extraction and derivatization in the lower chip. The extraction reservoir has a microfilter for the filtration of proteins contained in the sample on the downstream side of the reservoir. We set the filter dimensions to 50 $\times$ 50 μ m² because the extraterrestrial sample is assumed to be 10–200 μ m in diameter. The derivatization reservoir is used for the hydrolysis of proteins to amino acids and for fluorescent derivatization. The heating operations in the derivatization reservoir include closed and open heating operations. We

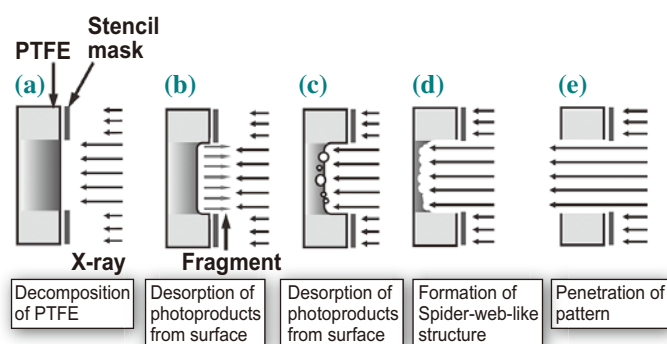


Fig. 1. Mechanism of direct dry etching of PTFE using SR.

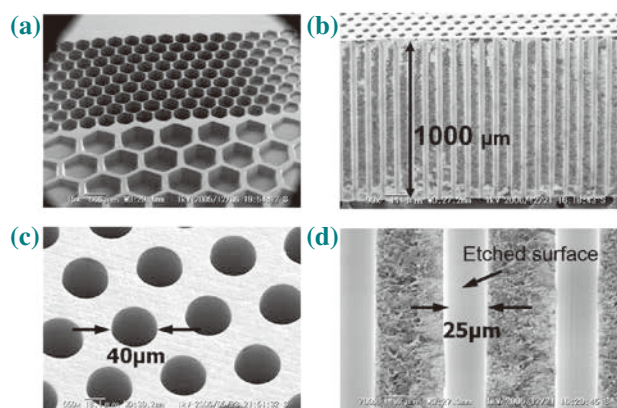


Fig. 2. SEM images of (a) honeycomb patterns, (b) cross section of through holes, (c) top view of through holes and (d) magnified cross section of through holes.

demonstrated both heating modes in a single reservoir by opening and closing the upper part of the reservoir. In addition, a filter structure is incorporated in the derivatization reservoir to prevent liquid leakage.

There have been no previous reports of the application of such a complex microstructure made of PTFE as a device for chemical analysis. This study is very important for the progress of science, technology and space development because our device has the potential to perform chemical analysis at the laboratory level at locations difficult to visit.

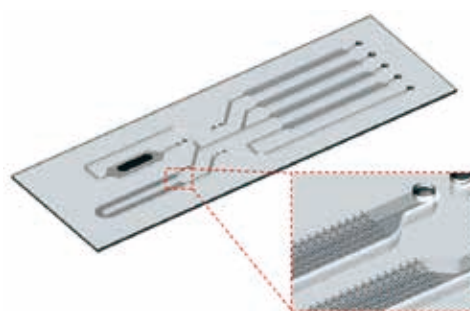


Fig. 3. Schematic diagram of amino acid analysis chip

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References

- [1] D.E. Brownlee *et al.*: J. Geophys. Res. **108** (2003) 8111.
- [2] Y. Zhang *et al.*: Appl. Phys. Lett. **67** (1995) 872.
- [3] Y. Utsumi *et al.*: Jpn. J. Appl. Phys. **44** (2005) 5500.
- [4] Y. Ukita *et al.*: Microsyst. Technol. **14** (2008) 1567.
- [5] H. Kido, I. Okada, H. Mita, A. Yamaguchi and Y. Utsumi: 10th International Workshop on High Aspect Ratio Micro and Nano System Technology (HARMNST), Berlin, 2013.

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